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A NOVEL RECEPTOR-TYPE TYROSINE KINASE AND USE THEREOF

Abstract:

The present invention relates generally to a novel receptor-type tyrosine kinase, to genetic sequences encoding same and to uses therefor. More particularly, the present invention contemplates a receptor-type tyrosine kinase having the following properties: (i) belongs to the Eph subfamily of RTKs as determined by conserved cysteine residues and fibronectin type III repeats; (ii) comprises protein tyrosine kinase catalytic domain motifs; and (iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79 % similarity thereto.

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A NOVEL RECEPTOR-TYPE TYROSINE KINASE AND USE THEREOF

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The present invention relates generally to a novel receptor-type tyrosine kinase, to genetic sequences encoding same and to uses therefor.

Bibliographic details of the publications numerically referred to in this specification are
10 collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or
15 variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The receptor tyrosine kinases (RTKs) form an important class of molecules involved in the
20 regulation of growth and differentiation of cells.

The RTKs are transmembrane molecules which transduce signals from the extracellular environment into the cytoplasm. They include well-studied regulators of cell proliferation and differentiation such as *c-kit* and the receptors for epidermal growth factor, platelet-derived
25 growth factor and macrophage colony-stimulating factor (1). Signalling is initiated when a cognate ligand binds to the RTK extracellular domain. This triggers a sequence of events resulting in the activation of an intracellular tyrosine kinase domain. Critical to this process is ligand-mediated receptor dimerization and reciprocal tyrosine phosphorylation by the dimerized molecules (2). Once their catalytic domain is activated, RTKs can bind and phosphorylate
30 specific intracellular proteins which act as second messengers.

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Eph was the first-isolated member of a new subfamily of RTKs (3). This group is distinguished by the sequential arrangement of a cysteine-rich region and two fibronectin-type-III repeats in the extracellular domain (4). At least 28 members have now been identified making this the largest subfamily of RTKs. They have been found in diverse species, including zebrafish (5),
5 frogs (6), chickens (4,7,8), mice (7,9,10-12,13), rats (14,15) and humans (3,16,17,18,19). Certain features of the expression pattern of the Eph subfamily suggest key functions during embryonic development. First, strong expression in the embryo is characteristic (4,7,8,9,11,20). Expression is generally down-regulated later in development, but often continues in the adult at a restricted number of tissue sites. Secondly, *in situ* hybridization and
10 immunolocalization studies have identified associations between the expression of specific Eph-subfamily molecules and particular events in morphogenesis. For example, Eck is transiently expressed in cells adjacent to the primitive streak during gastrulation and later its transcripts are found in specific rhombomeres of the developing hindbrain and in the ectoderm of the second and third branchial arches (21).

15

The expression of Nuk protein on growing peripheral nervous system axons, which disappears when the axons have ceased migrating, and the segment-restricted pattern of Nuk and Sek expression during hindbrain morphogenesis, are other examples (9,11). Preferential expression at interfaces between embryonic cell populations and in intercellular junctions has led to the
20 suggestion that Eph-subfamily molecules influence embryonic differentiation and cellular migration by interactions involving direct cell-cell contact (11,21). The recent finding that ligands for some members of this group are cell membrane bound supports this notion (22-25). Eph-subfamily kinases are also significant because of a potential role in oncogenesis. Eph and Erk are over expressed in some epithelial tumor cell lines and carcinomas (3,26), while Hek
25 overexpression occurs sporadically in leukemia (32). Furthermore, artificial overexpression of Hek or Eph in NIH-3T3 cells resulted in a transformed phenotype, as evidenced by the ability to form colonies in agar and tumors in nude mice (27). These molecules may also be involved in tumor progression. In transgenic models of murine mammary cancer, overexpression of the Eph-subfamily members Myk-1 and Myk-2 correlated with the development of poorly-
30 differentiated and invasive tumors (13).

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Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst (28). They are undifferentiated and totipotent. When grown *in vitro* in the absence of leukemia inhibitory factor (LIF), they develop into embryoid bodies containing cells committed to a variety of tissue lineages (29). This system provides a convenient model of very early embryonic development, which is difficult to study *in vivo* because of problems in harvesting preimplantation embryos and the small amounts of tissue involved.

In work leading up to the present invention, the inventors considered that RTKs expressed by ES cells and embryoid bodies are likely to be involved in the initial differentiation and organization of embryonic tissues. To analyse this further, the inventors used reverse transcriptase (RT) -mediated polymerase chain reaction (PCR) [RT-PCR] to identify Eph subfamily RTKs in ES cells. In accordance with the present invention, the inventors have identified a novel RTK member from the Eph-subfamily.

Accordingly, one aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a novel member of the Eph subfamily of RTKs or a derivative, homologue or chemical analogue thereof.

More particularly, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an RTK or a derivative, homologue or chemical analogue thereof having the following characteristics:

- (i) belongs to the Eph subfamily of RTKs as determined by conserved cysteine residues and fibronectin type III repeats;
- (ii) comprises protein tyrosine kinase catalytic domain motifs; and
- (iii) comprises an amino acid sequence substantially as set forth in Figure 2 [SEQ ID NO:2] or having at least about 79% similarity to all or part thereof.

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Preferably, the novel RTK of the present invention is of animal or mammalian origin. Preferred mammals include but are not limited to humans, primates, livestock animals (e.g. sheep, cows, horses, pigs, donkeys), laboratory test animals (e.g. rabbits, mice, rats, guinea pigs), companion animals (e.g. dogs, cats) or captive wild animals (e.g. foxes, kangaroos, deer).

- 5 Preferred non-mammalian animals include fish and birds. The present invention is particularly exemplified herein by reference to a novel RTK of murine origin but this is done with the understanding that the present invention extends to all animal and mammalian homologues of the novel murine Eph-subfamily RTK and in particular a human form of the RTK. Hereinafter, the novel RTK of the present invention is referred to as "Esk" for "embryonic stem cell kinase".

10

The present invention extends to derivatives, homologues and chemical analogues of Esk, which Esk has an amino acid sequence set forth in Figure 2 [SEQ ID NO:2]. Derivatives include single or multiple amino acid substitutions, deletions and/or additions to the sequence and encompass mutants, part and fragments thereof. The term "derivative" also encompasses
15 soluble or solubilized or otherwise secreted forms of the Esk molecule. Derivatives also encompass chimeric molecules comprising Esk or a derivative, homologue, or analogue thereof and at least one other molecule such as another receptor or ligand.

- Homologues include novel Esks of animal or mammalian origin having at least about 79%,
20 more preferably at least about 85%, even more preferably at least about 90% and still more preferably at least about 95% or above sequence similarity to the amino acid sequence set forth in Figure 2 [SEQ ID NO:2]. Preferred homology comparisons are done between coding regions or 3' or 5' regulatory regions or particularly conserved regions.

- 25 Analogues of Esk contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptides or their analogues. Such analogues may also provide stability to molecule administered *in vivo* or for manipulation of molecules *in vitro*.

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Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with NaBH_4 ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups
5 with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with NaBH_4 .

The guanidine group of arginine residues may be modified by the formation of heterocyclic
10 condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

15 Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol
20 and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form
25 a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

30 Examples of the incorporation of unnatural amino acids and derivatives during polypeptide

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synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated for use
5 in accordance with the present invention is given in Table 1.

TABLE 1

| Non-conventional 5 amino acid | Code | Non-conventional amino acid | Code |
|---|------|--------------------------------|--------|
| α -aminobutyric acid | Abu | L-N-methylalanine | Nmala |
| α -amino- α -methylbutyrate | Mgab | L-N-methylarginine | Nmarg |
| aminocyclopropane- | Cpro | L-N-methylasparagine | Nmasn |
| 10 carboxylate | | L-N-methylaspartic acid | Nmasp |
| aminoisobutyric acid | Aib | L-N-methylcysteine | Nmcys |
| aminonorbornyl- | Norb | L-N-methylglutamine | Nmgln |
| carboxylate | | L-N-methylglutamic acid | Nmglu |
| cyclohexylalanine | | Chexa L-N-methylhistidine | Nmhis |
| 15 cyclopentylalanine | Cpen | L-N-methylisoleucine | Nmile |
| D-alanine | Dal | L-N-methylleucine | Nmleu |
| D-arginine | Darg | L-N-methyllysine | Nmlys |
| D-aspartic acid | Dasp | L-N-methylmethionine | Nmmet |
| D-cysteine | Dcys | L-N-methylnorleucine | Nmnle |
| 20 D-glutamine | Dgln | L-N-methylnorvaline | Nmnva |
| D-glutamic acid | Dglu | L-N-methylornithine | Nmorn |
| D-histidine | Dhis | L-N-methylphenylalanine | Nmphe |
| D-isoleucine | Dile | L-N-methylproline | Nmpro |
| D-leucine | Dleu | L-N-methylserine | Nmser |
| 25 D-lysine | Dlys | L-N-methylthreonine | Nmthr |
| D-methionine | Dmet | L-N-methyltryptophan | Nmtrp |
| D-ornithine | Dorn | L-N-methyltyrosine | Nmtyr |
| D-phenylalanine | Dphe | L-N-methylvaline | Nmval |
| D-proline | Dpro | L-N-methylethylglycine | Nmetg |
| 30 D-serine | Dser | L-N-methyl-t-butylglycine | Nmtbug |

| | | | | |
|----|----------------------------------|--------|---|--------|
| | D-threonine | Dthr | L-norleucine | Nle |
| | D-tryptophan | Dtrp | L-norvaline | Nva |
| | D-tyrosine | Dtyr | α -methyl-aminoisobutyrate | Maib |
| | D-valine | Dval | α -methyl- γ -aminobutyrate | Mgab |
| 5 | D- α -methylalanine | Dmala | α -methylcyclohexylalanine | Mchex |
| | D- α -methylarginine | Dmarg | α -methylcyclopentylalanine | Mcpn |
| | D- α -methylasparagine | Dmasn | α -methyl- α -naphthylalanine | Manap |
| | D- α -methylaspartate | Dmasp | α -methylpenicillamine | Mpen |
| | D- α -methylcysteine | Dmcys | N-(4-aminobutyl)glycine | Nglu |
| 10 | D- α -methylglutamine | Dmgln | N-(2-aminoethyl)glycine | Naeg |
| | D- α -methylhistidine | Dmhis | N-(3-aminopropyl)glycine | Norn |
| | D- α -methylisoleucine | Dmile | N-amino- α -methylbutyrate | Nmaabu |
| | D- α -methylleucine | Dmleu | α -naphthylalanine | Anap |
| | D- α -methyllysine | Dmlys | N-benzylglycine | Nphe |
| 15 | D- α -methylmethionine | Dmmet | N-(2-carbamylethyl)glycine | Ngln |
| | D- α -methylornithine | Dmorn | N-(carbamylmethyl)glycine | Nasn |
| | D- α -methylphenylalanine | Dmphe | N-(2-carboxyethyl)glycine | Nglu |
| | D- α -methylproline | Dmpro | N-(carboxymethyl)glycine | Nasp |
| | D- α -methylserine | Dmser | N-cyclobutylglycine | Ncbut |
| 20 | D- α -methylthreonine | Dmthr | N-cycloheptylglycine | Nchep |
| | D- α -methyltryptophan | Dmtrp | N-cyclohexylglycine | Nchex |
| | D- α -methyltyrosine | Dmty | N-cyclodecylglycine | Ncdec |
| | D- α -methylvaline | Dmval | N-cylcododecylglycine | Ncdod |
| | D-N-methylalanine | Dnmala | N-cyclooctylglycine | Ncoct |
| 25 | D-N-methylarginine | Dnmarg | N-cyclopropylglycine | Ncpro |
| | D-N-methylasparagine | Dnmasn | N-cycloundecylglycine | Ncund |
| | D-N-methylaspartate | Dnmasp | N-(2,2-diphenylethyl)glycine | Nbhm |
| | D-N-methylcysteine | Dnmcys | N-(3,3-diphenylpropyl)glycine | Nbhe |
| | D-N-methylglutamine | Dnmgln | N-(3-guanidinopropyl)glycine | Narg |
| 30 | D-N-methylglutamate | Dnmglu | N-(1-hydroxyethyl)glycine | Nthr |

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| | | | | |
|----|----------------------------------|---------|---|--------|
| | D-N-methylhistidine | Dnmhis | N-(hydroxyethyl)glycine | Nser |
| | D-N-methylisoleucine | Dnmile | N-(imidazolylethyl)glycine | Nhis |
| | D-N-methylleucine | Dnmleu | N-(3-indolylyethyl)glycine | Nhtrp |
| | D-N-methyllysine | Dnmlys | N-methyl- γ -aminobutyrate | Nmgabu |
| 5 | N-methylcyclohexylalanine | Nmchexa | D-N-methylmethionine | Dnmmt |
| | D-N-methylornithine | Dnmorn | N-methylcyclopentylalanine | Nmcpen |
| | N-methylglycine | Nala | D-N-methylphenylalanine | Dnmphe |
| | N-methylaminoisobutyrate | Nmaib | D-N-methylproline | Dnmpro |
| | N-(1-methylpropyl)glycine | Nile | D-N-methylserine | Dnmser |
| 10 | N-(2-methylpropyl)glycine | Nleu | D-N-methylthreonine | Dnmthr |
| | D-N-methyltryptophan | Dnmtrp | N-(1-methylethyl)glycine | Nval |
| | D-N-methyltyrosine | Dnmtyr | N-methyl-naphthylalanine | Nmanap |
| | D-N-methylvaline | Dnmval | N-methylpenicillamine | Nmpen |
| | γ -aminobutyric acid | Gabu | N-(<i>p</i> -hydroxyphenyl)glycine | Nhtyr |
| 15 | L- <i>t</i> -butylglycine | Tbug | N-(thiomethyl)glycine | Ncys |
| | L-ethylglycine | Etg | penicillamine | Pen |
| | L-homophenylalanine | Hphe | L- α -methylalanine | Mala |
| | L- α -methylarginine | Marg | L- α -methylasparagine | Masn |
| | L- α -methylaspartate | Masp | L- α -methyl- <i>t</i> -butylglycine | Mtbug |
| 20 | L- α -methylcysteine | Mcys | L-methylethylglycine | Metg |
| | L- α -methylglutamine | Mgln | L- α -methylglutamate | Mglu |
| | L- α -methylhistidine | Mhis | L- α -methylhomophenylalanine | Mhphe |
| | L- α -methylisoleucine | Mile | N-(2-methylthioethyl)glycine | Nmet |
| | L- α -methylleucine | Mleu | L- α -methyllysine | Mlys |
| 25 | L- α -methylmethionine | Mmet | L- α -methylnorleucine | Mnle |
| | L- α -methylnorvaline | Mnva | L- α -methylornithine | Morn |
| | L- α -methylphenylalanine | Mphe | L- α -methylproline | Mpro |
| | L- α -methylserine | Mser | L- α -methylthreonine | Mthr |
| | L- α -methyltryptophan | Mtrp | L- α -methyltyrosine | Mtyr |

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| | | | |
|----------------------------|-------|-----------------------------|--------|
| L- α -methylvaline | Mval | L-N-methylhomophenylalanine | Nmhphe |
| N-(N-(2,2-diphenylethyl) | Nnbhm | N-(N-(3,3-diphenylpropyl) | Nnbhe |
| carbamylmethyl)glycine | | carbamylmethyl)glycine | |
| 1-carboxy-1-(2,2-diphenyl- | Nmbc | | |
| 5 ethylamino)cyclopropane | | | |

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional
 10 crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with $n=1$ to
 $n=6$, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which
 usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group
 specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH).

In addition, peptides can be conformationally constrained by, for example, incorporation of
 15 C_α and N_ϵ -methylamino acids, introduction of double bonds between C_α and C_β atoms of
 amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds
 such as forming an amide bond between the N and C termini, between two side chains or
 between a side chain and the N or C terminus.

20 Particularly useful derivatives contemplated by the present invention are soluble forms of the
 Esk receptor. A soluble receptor is also referred to herein as a secreted Esk protein and is
 most preferably in recombinant form. Soluble Esk molecules are useful reagents for ligand
 isolation, as antagonists of Esk-ligand interaction, as a substrate for antibody production which
 antibodies are in turn useful diagnostic reagents.

25

Accordingly, another aspect of the present invention provides a secreted recombinant Esk
 protein or derivative thereof comprising conserved cysteine residues and fibronectin type III
 repeats and a portion of the amino acid sequence substantially set forth in SEQ ID NO:2 or
 having at least 79% homology thereto which is not part of the membrane bound region of the
 30 corresponding anchored receptor. Preferably, the amino acid sequence of the secreted

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recombinant Esk is encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:2 or having at least 82% homology thereto.

In a particularly preferred embodiment, the nucleic acid molecule comprises a nucleotide
5 sequence (or a complementary form thereof) substantially as set forth in Figure 2 [SEQ ID NO:1] or having at least about 82% similarity to all or part thereof or is capable of hybridising to the sequence set forth in SEQ ID NO:1 or a complementary form thereof under low stringency conditions.

- 10 Preferred percentage nucleotide similarities include at least about 84%, more preferably at least about 90% and even more preferably at least about 95% or above. The nucleic acid molecules of the present invention include single or multiple nucleotide substitutions, deletions and/or additions to the nucleotide sequence set forth in Figure 2 [SEQ ID NO:1] and mutants, parts and fragments thereof, which are all encompassed by the term "derivative" of the
15 nucleotide sequence set forth in Figure 2 [SEQ ID NO:2].

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions.

- 20 Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide
25 and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The nucleic acid molecules of the present invention are preferably carried by a vector molecule and more particularly an expression vector. Preferred expression vectors direct
30 expression in mammalian, insect and/or bacterial cells. The present invention also extends to

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cells carrying the recombinant nucleic acid molecules of the present invention.

Preferred nucleic acid molecules are DNA and more preferably cDNA.

5 The present invention is also directed to a recombinant Esk polypeptide having the following characteristics:

- (i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;
- (ii) comprises protein tyrosine kinase catalytic domain motifs; and
- 10 (iii) comprises an amino acid sequence substantially as set forth in Figure 2 [SEQ ID NO:2] or having at least about 79% similarity to all or part thereof.

The recombinant Esk is preferably in isolated form meaning that a composition comprises at least about 20%, more preferably at least about 30%, still more preferably at least about 40-
15 50%, even more preferably at least about 60-70% and yet even more preferably at least about 80-90% or above of Esk as determined by activity, molecular weight, or immunological reactivity. The preparation may also be sequencably pure or of a purity suitable for use in a pharmaceutical composition.

20 The present invention extends to the ligand(s) of Esk and to agonists and antagonists of Esk-ligand interaction. Agonists and antagonists may be, for example, antibodies or derivatives of the Esk or derivatives of the ligand. Derivatives of Esk or its ligand include soluble or solubilised forms thereof. Reference herein to "Esk" includes both anchored forms (ie. membrane bound forms) of the receptor as well as soluble (ie. secreted) forms of the receptor.

25

Modulating expression of Esk may have important potential in therapeutic regimens for the treatment or prophylaxis of cancers caused or exacerbated by aberrations in Esk or aberrations in Esk-ligand interaction. This will be particularly important for the treatment of mucositis.

This condition remains the major adverse effect of chemotherapy and radiotherapy of
30 malignant disease. As Esk is expressed in all epithelial tissues, modulation of Esk-ligand

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interactions may have therapeutic applications for skin (such as following burns, abrasions, eczema and other forms of skin wounding leading to skin loss or damage); hair (hair loss or abnormal hair growth); corneal ulcers (injury, infection, Sjogrens syndrome and related autoimmune diseases; mucositis (mouth, oesophagus, stomach, small and large bowel);
5 infective ulcerative colitis and other non-infective inflammatory enteritides; peptide ulcers; oesophageal reflux; Sjogrens syndrome and related autoimmune diseases; infection of other mucous surfaces (eg. vaginitis and vulvitis); Sjogrens syndrome and related autoimmune diseases; infection of the lung (eg. shock lung, inhalation of noxious fumes, infection); liver (eg. regeneration after viral illness or toxic damage); pancreatitis; urological disease involving
10 tubules, pelvicalyceal system, ureters, bladder or urethra and salivary glands.

Accordingly, the present invention contemplates a method for modulating Esk-ligand interaction in an animal, said method comprising administering to said animal a modulating effective amount of an agonist or antagonist of Esk-ligand interaction. The term "modulating"
15 includes facilitating Esk-ligand interaction or inhibiting, reducing or otherwise interfering with Esk-ligand interaction. Either form of modulation may be required depending on, for example, the type of treatment such as the treatment of cancer or the promotion or inhibition of cell apoptosis.

20 The present invention, therefore, contemplates a pharmaceutical composition comprising an Esk-ligand interaction modulating effective amount of an agonist or antagonist of Esk-ligand interaction and one or more pharmaceutically acceptable carriers and/or diluents.

The formation of pharmaceutical compositions is generally known in the art and reference can
25 conveniently be made to Remington's Pharmaceutical Sciences, 17th end., Mack Publishing Co., Easton, Pennsylvania, USA.

The active ingredients of a pharmaceutical composition comprising the Esk agonists or antagonists or their derivatives are contemplated herein to exhibit excellent therapeutic
30 activity, for example, in modulating Esk-ligand interaction when administered to an animal

in an amount which depends on the particular case. For example, from about 0.5 μg to about 20 mg per kilogram of body weight per day may be administered. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly or monthly, or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The active compounds may be administered in any convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or by implanting (eg using slow release molecules), topical administration or following or during surgery or biopsy or other invasive procedure. Depending on the route of administration, the active ingredients which comprise the Esk agonists or antagonists or chemical analogues thereof may be required to be coated in a material to protect said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredients. In order to administer Esk agonists or antagonists by other than parenteral administration, they will be coated by, or administered with, a material to prevent its inactivation. For example, homologues may be administered in an adjuvant, co-administered with enzyme inhibitors or in liposomes. Adjuvants contemplated by the present invention include, but are not limited to, cytokines (e.g. interferons) as well as resorcinols, non-ionic surfactants such as polyoxyethelene oleyl ether and n-hexadecyl polyethylene ether.

20 The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

25 The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

30

- 15 -

example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of
5 superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying
10 absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by
15 incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation of vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-
20 filtered solution thereof.

When the active agonists or antagonists or chemical analogues thereof are suitably protected as described above, the active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin
25 capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and
30 preparations may, of course, be varied and may conveniently be between 5 to about 80% of

the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that a oral dosage unit form contains between about 0.1 μ g and 2000 mg of active compound.

5

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may
10 be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound,
15 sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

20

A pharmaceutically acceptable carrier and/or diluent includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the
25 active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

Its is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to
30 physically discrete units suited as unitary dosages for the mamallian subjects to be treated;

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each unit coating a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic affect to be achieved, and (b) the limitations inherent in the art of compound such a active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions coating supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The animal to be treated is preferably a mammal such as a human, primate, livestock animal, laboratory test animal, companion animal or captive wild animal. Most preferably, the animal is human.

The present invention further extends to antibodies to the Esk molecules herein described. The antibodies may be monoclonal or polyclonal. Antibodies to the Esk molecules of the present invention are useful as therapeutic agents in modulating Esk-ligand interaction or as diagnostic agents to assay for Esk molecules or Esk-ligand interaction. Assay techniques are well known in the art and include, for example, sandwich assays and ELISA.

Accordingly, another aspect of the present invention contemplates a method for assaying for Esk expression on a cell, said method comprising contacting a biological sample containing cells putatively expressing Esk with an Esk-binding effective amount of an antibody thereto

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for a time and under conditions sufficient for said antibody to bind to said Esk and then detecting said Esk-antibody binding.

The presence of Esk on a cell can be detected using a wide range of immunoassay techniques
5 such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. This includes both single-site and two-site, or "sandwich", assays of the non-competitive types, as well as in the traditional competitive binding assays. Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by
10 the present invention. Briefly, in a typical forward assay, and Esk antibody is immobilised onto a solid substrate to form a first complex and the sample containing cells to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an Esk-antibody secondary complex, an antibody, labelled with a receptor molecule capable of producing a detectable signal and
15 specific to another antigen to the cell, is then added and incubated, allowing time sufficient for the formation of a tertiary complex of Esk-antibody-labelled antibody. Any unreacted material is washed away, and the presence of the first antibody is determined by observation of a signal produced by the reporter molecule on the second antibody. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing
20 with a control sample containing known amounts of hapten. Variations of the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and then added simultaneously to the bound antibody.

25

These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. Alternatively, a labelled Esk antibody may be added directly to the sample of cells and the reporter molecule detected.

30 The solid substrate is typically glass or a polymer, the most commonly used polymers being

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cellulose, polyacrylamide, nylon, polystyrene polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytical identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay is either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art.

Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates which yield a fluorescent product.

20

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescent observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such

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as radiosotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

- 5 The present invention further extends to genetic molecules derived from an *Esk* gene useful as probes, antisense or sense molecules for diagnostic or therapeutic situations.

The present invention is further described by the following non-limiting Figures and/or Examples.

10

Reference herein to "similarity" in relation to amino acid or nucleotide acid or nucleotide sequences has substantially the same meaning as "homology" and "identify".

In the Figures:

15

Figure 1 is a diagrammatic representation showing the position of the four degenerate oligonucleotide primers (P1-P4) used for RT-PCR. The relatively-conserved peptide motifs on which the primers were based are shown above a schematic representation of the basic domain structure of Eph-subfamily molecules. The particular amino acid sequences used
20 in the figure are from *eph*. P1 and P2 were sense primers: P3 and P4 were antisense primers. Their sequences were as follows:

P1, 5'-GTAGGCATGCAAGGAGAC(AC)TT(CT)AACC-3' [SEQ ID NO:3];

P2, 5'-GCGATGATCAT(CG)AC(AGT)GA(AG)TA(CT)ATGG-3' [SEQ ID NO:4];

P3, 5'-GTAGGAATTCCA(CGT)ACATC(AG)CT(AG)GC-3' [SEQ ID NO:5];

25 P4, 5'-CCA(TA)A(AG)CTCCA(CT)ACATC(AG)CT-3' [SEQ ID NO:6].

TMD denotes the transmembrane domain.

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Figure 2 is a representation showing the nucleotide and deduced amino acid sequence of Esk. The signal peptide and transmembrane domain are boxed. Conserved cysteine residues in the extracellular domain are circled and two fibronectin type III repeats are in a striped box. In the catalytic domain, arrowheads indicate the highly-conserved Gly-X-Gly-X-X-Gly motif and a dot marks the invariant lysine residue. Two motifs associated with substrate specificity for tyrosine are underlined. The stop codon which terminates the coding region is indicated by an asterisk.

Figure 3 is a photographic representation of expression of Esk. Northern blots of poly(A)⁺ RNA from day-12 mouse embryo and the adult mouse tissues shown were hybridized to a ³²P-labelled probe derived from clone 35C15. This was then stripped from the filters and hybridization to a GAPDH probe was performed. The positions of RNA size markers are indicated to the left of the blots.

Figure 4 is a photographic representation showing Northern blot of ES cell, embryoid body and embryonic fibroblast poly (A)⁺ RNA hybridized to ³²P-labelled probes synthesized from Esk,, Mek4 and Eck cDNA. Probes were stripped from the filter between reprobing. Hybridization to a GAPDH probe was performed last. The sizes of transcripts are indicated to the right of the figure.

20

Figure 5 is a diagrammatic representation of all Eph-subfamily clones isolated from ES cells using RT-PCR aligned with the full-length cDNA clone derived from a λZAP library. Numbers represent nucleotides in the Esk full length sequence. ECD, extracellular domain; ICD, intracellular domain.

25

Figure 6 is a representation showing a plot of grains after scoring approximately 130 Chromosomes 6, showing probable localisation of Esk to bands B1-B2. Grains scored from C57BL and BALB/c mice are represented with solid and open dots respectively.

Figure 7 is a photographic representation of an analysis of the relationship between Esk and

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Eph by Southern hybridization. A mouse genomic Southern blot was prepared using DNA digested with the restriction enzymes shown. This was initially hybridized in 40% formamide to a probe derived from the catalytic domain of human Eph (A). The membrane was then stripped and rehybridized in 50% formamide to a probe derived from equivalent sequence in mouse Esk(B). The position of DNA size markers is indicated at left.

Figure 9 is a photographic representation showing *in situ* analysis of Esk expression in whole-mounted embryoid bodies (A) and day 9.5 mouse embryos (B). Embryoid bodies were differentiated *in vitro* from ES cells by culturing without feeder cells in LIF-deficient medium, for 7-10 days. Sense and antisense digoxigenin-labelled riboprobes were synthesized from cDNA fragments of Esk and hybridized to the whole-mounts. Bound probe was detected using alkaline phosphate-conjugated anti-digoxigenin Fab fragments and staining to detect enzyme activity. Left panels show results of hybridization with sense-control and right panels with Esk antisense probe.

15

Figure 9 is a photographic representation of Esk expression in sections of embryoid bodies and selected mouse tissues. Results of hybridizations with sense-control probe are shown in the left panels and with Esk antisense probe in the middle and right panels. (A) Embryoid bodies differentiated *in vitro*. (B) Adult thymus. (C) Adult renal cortex. (D) Day 18 embryo skin. Abbreviations: LP, low power; HP, high power.

20

Figure 10 is a photographic representation showing binding of ligand to Esk chip in a biosense assay for binding to a potential Erk ligands. (L1 to L7) controls a FC, L3-FLAG, L7-FLAG and binding to HEK.

25

Single and three letter abbreviations for amino acid residues are used in the specification and are defined in Table 2.

TABLE 2

| Amino Acid | Three-letter Abbreviation | One-letter Symbol |
|---------------|------------------------------|----------------------|
| 10 | | |
| Alanine | Ala | A |
| Arginine | Arg | R |
| Asparagine | Asn | N |
| Aspartic acid | Asp | D |
| 15 | | |
| Cysteine | Cys | C |
| Glutamine | Gln | Q |
| Glutamic acid | Glu | E |
| Glycine | Gly | G |
| Histidine | His | H |
| 20 | | |
| Isoleucine | Ile | I |
| Leucine | Leu | L |
| Lysine | Lys | K |
| Methionine | Met | M |
| Phenylalanine | Phe | F |
| 25 | | |
| Proline | Pro | P |
| Serine | Ser | S |
| Threonine | Thr | T |
| Tryptophan | Trp | W |
| Tyrosine | Tyr | Y |
| 30 | | |
| Valine | Val | V |
| Any residue | Xaa | X |

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EXAMPLE 1

ES CELL CULTURES

The murine 129/Sv-derived ES cell line, W9.5 was routinely passaged on underlays of
5 irradiated embryonic fibroblasts in Dulbecco's modified Eagle medium supplemented with
1000 units/ml of LIF (AMRAD Operations Pty Ltd, Melbourne, Victoria, Australia), 10^{-4}
M 2-mercaptoethanol and 15% v/v fetal calf serum. Cultures were incubated in a 10% v/v
CO₂ atmosphere at 37°C. In preparation for the studies described below, ES cells were
subcultured into delatinized flasks and four passages without a feeder layer were performed
10 to deplete the embryonic fibroblasts. In some of these cultures, LIF was withdrawn 11 days
prior to harvesting the cells, to allow differentiation into embryoid bodies (29). Control
cultures of embryonic fibroblasts alone were also performed.

EXAMPLE 2

RT-PCR

15

Prior to RNA extraction, cultures of undifferentiated ES cells were disrupted with trypsin
and washed in phosphate buffered saline. Cell pellets were resuspended in guanidine
isothiocyanate denaturing buffer and total RNA was extracted using organic solvents (30).
20 cDNA was then synthesized using 1 µg of total RNA, an aoligo(dT) primer and AMV
reverse transcriptase (Promega). Four degenerate PCR primers were derived from three
regions of sequence which are relatively conserved across the Eph subfamily (18)[Figure1].
Sense primers P1or P2 were used with antisense primer P4 to amplify the Es cell cDNA.
Reactions were carried out in a 30µl volume containing 50 mM KC1, 10mM Tris.HCl (pH
25 8.3), 1.25 mM MgCl₂, 0.2 mM each dNTP, 2.5 units of Taq polymerase (Perkin Elmer),
30 pmol of each promer and 3µl of the ES cell cDNA synthesis reaction. In another
experiment, mRNA was directly extracted from ES cells with oligo9dT)-coated magnetic
beads (Dyna; Oslo, Norway) and cDNA was synthesized from 1 µg of mRNA using an
oligo(dT) primer and Superscript II reverse transcriptase (Life Technologies). Half of the
30 reverse transcription reaction was amplified using primers P1 and P4, with other reaction

- 25 -

conditions identical to those described above. PCR products were electrophoretically separated, purified with the Geneclean II Kit (Bio 101 Inc) and then subjected to a second round of PCR. Products initially amplified using primers P1 and P4 were reamplified with the same primers, while those amplified with P2 and P4 were reamplified with P2 and P3.

- 5 All reactions were carried out in a PTC-100 Programmable Thermal Controller (MJ Research Inc) employing programs specific to each primer combination, as follows: 1min at 95°C, 2 min at 70°C and 3 min at 72°C for 30 cycles (primers P1 and P4); 1 min at 95°C, 1 min at 51°C and 1 min at 72°C for 35 cycles (primers P2 and P4); 1 min at 95°C, 1 min at 41°C and 1 min at 72°C for 35 cycles (primers P2 and P3).

10

EXAMPLE 3 CLONING AND SEQUENCING

Reamplified, gel-purified PCR fragments were cloned into the *Sma*I site of pUC18 using the SureClone Ligation Kit (Pharmacia). A ³²P-labelled probe was synthesized from a cloned
15 cDNA fragment of Esk. This was used to screen 8 x 10⁵ clones from a mouse liver cDNA library constructed in λZAP. (Stratagene), under high stringency conditions. Secondary screening of selected primary positive plaques was used to identify clones with the largest inserts. Recombinant clones were sequenced with the Taq DyeDeoxy terminator Cycle Sequencing Kit (Applied Biosystems), using a Perkin Elmer GeneAmp PCR System 2400
20 to perform the sequencing reactions and an Applied Biosystems 373 DNA Sequencer for their subsequent analysis. Sequences were compared against sequence databank entries with the FASTA sequence analysis program. A diagrammatic representation of Esk clones is shown in Figure 5.

25

EXAMPLE 4 NORTHERN BLOTS

Oligo(dT)-cellulose (Pharmacia) as used to isolate poly(A)⁺ RNA from total derived from ES cells, embryoid bodies differentiated *in vitro* from ES cells. Embryonic fibroblasts, day-12 mouse embryos and adult mouse tissues. The poly(A)-selected samples (5μg of each)
30 were electrophoresed through 1.2% w/v agarose gels containing 2.2 M formaldehyde and

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transferred to nylon membranes (Zeta-Probe, Bio-Rad). The resulting Northern blots were probed with cDNA inserts from selected ES cell recombinants cloned by the methods described earlier. Sequence analysis (see below) had revealed that a partial cDNA of murine Eck (20) was present in the clone designated 35C4 and that Mek4 (7) cDNA was present in clone 35C11. Furthermore, an apparently novel sequence was obtained in clone 35C15. Inserts were digested from these three clones with the restriction enzymes *EcoRI* and *XbaI*, and used as templates for synthesizing ³²P-labelled probes with the Prime-It II Random Primer Labelling Kit (Stratagene). A labelled probe was also made from glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. The Northern blots were initially hybridized to the 35C15 probe. Subsequently the membrane containing RNA from ES cells, embryoid lodies and embryonic fibroblasts was re-probed with the Eck and Mek4 probes. Between each re-probing, hybridized probe was stripped from the membrane by pouring on boiling 0.1% w/v SDS and cooling to room temperature. Exposure to X-ray film overnight confirmed effective removal of the probe. Hybridisation to the GAPDH probe was performed last. In all cases, hybridisations took place in 50% v/v formamide at 42°C and washes were performed under stringent conditions, with the final wash at 65°C in 0.1 x SSC and 0.1% w/v SDS (SSC is 0.15 M NaCl and 0.015 M Na citrate, pH 7.6). Autoradiographs were exposed at -70°C.

20

EXAMPLE 5

SOUTHERN ANALYSIS

A ³²P-labelled Esk probe was synthesised from a PCR fragment amplified from Esk cDNA using degenerate kinase-domain primers described in Example 2. The fragment included sequence from bases 2098-2437 of Esk. Probe was hybridized to a genomic Southern blot made using standard techniques with DNA extracted from the murine embryonic stem cell line W9.5 and digested with the panel of enzymes shown in Figure 7. Hybridisation was performed in 50% formamide, 10x Denhardt's solution (35), 50mM Tris HCl (pH7.5), 1.0 M NaCl, 2.24 mM tetrasodium pyrophosphate, 1% w/v SDS, 10% w/v dextran sulfate and 0.1 mg/ml sheared heat-denatured herring sperm DNA. The final wash was in 0.1xSSC/0.1% w/v SDS at 65°C (SSC is 0.15 M sodium chloride, 0.015 M sodium citrate,

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pH 7.6). An autoradiograph was exposed at 70°C for eight days> The filter was subsequently stripped using 0.4 M sodium hydroxide and removal of the probe was confirmed by exposure to film overnight.

- 5 A ³²P-labelled Eph probe was synthesised from a cloned cDNA fragment isolated by RT-PCR from human bone marrow cDNA, using the same degenerate kinase-domain primers that generated the Esk probe. The cloned cDNA included sequence from bases 2212-2553 of human Eph. Probe was hybridized to the stripped Southern blot, with hybridization conditions and washes identical to those used with the Esk probe. An autoradiograph was exposed at
10 -70°C for seven days.

EXAMPLE 6

In vitro DIFFERENTIATION OF EMBRYOID BODIES

- 15 ES cells from the W9.5 line were induced to undergo differentiation by passaging them in bacterial-grade Petri dishes without an embryonic fibroblast feeder layer and in the absence of LIF. Embryoid bodies were harvested after 7-10 days of development (39).

EXAMPLE 7

20 PREPARATION OF WHOLE-MOUNTED AND SECTIONED TISSUE

- CBA mouse embryos for whole-mount studies were collected 8-10 days following observation of the coital plug and a more accurate age was determined by reference to a standard atlas of mouse development (40). After opening the brain ventricles and heart chambers to prevent
25 trapping of probe, the embryos were fixed in 4% v/v paraformaldehyde. Embryoid bodies for sections were set in 1% w.v agar, to which Bouin's fixative was added. Other tissues for sectioning, including day 18 CBA embryos and thymus, liver, kidney and adrenal gland from six-week-old BALB/c mice, were dissected free and fixed in 4% v/v paraformaldehyde. Sections were cut at a thickness of 5 µm after dehydration in ethanol and embedding in
30 paraffin.

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EXAMPLE 9

In situ HYBRIDIZATION PROCEDURE

5 The hybridization of digoxigenin-labelled riboprobes to whole-mounted specimens was performed using methods described in detail elsewhere (41), except that 2mM levamisole was included in the washes prior to colour development to inactivate endogenous alkaline phosphatases. Tissue sections were first soaked in histolene (Histo Labs) to remove paraffin and then rehydrated through decreasing concentrations of methanol in PBS. Refixation in 4%
10 v/v paraformaldehyde and 0.2% v/v glutaraldehyde was then performed. Subsequent steps were similar to those of the whole-mount procedure.

EXAMPLE 10

Eph-SUBFAMILY MOLECULES EXPRESSED BY ES CELLS

15 Primers P2 and P4 were expected to amplify approximately 350 bp from the catalytic domain of Eph-subfamily molecules. In contrast, it was anticipated that primers P1 and P4 would amplify about 2.1 kb, including much of the extracellular and intracellular domains (Figure 1). RT-PCR was initially performed on total RNA derived from ES cells. Reactions using primers P2 and P4 amplified a band of the expected size, which reamplified with primers P2
20 and P3. However, no PCR product of the anticipated size was observed when primers P1 and P4 were used. Subsequently, when magnetically-separated poly(A)⁺ RNA was substituted for total RNA, reactions with primers P1 and P4 successfully amplified a 2.1 kb product. Cloning of the 350 bp product resulted in four recombinants containing Eph-subfamily sequences (Table 3). These were highly homologous (>97%) to either Sek (9), Nuk (11) or Eck (20) -
25 members of the Eph subfamily previously isolated from murine sources. When the 2.1 kb product was cloned, five recombinants containing sequences of this subfamily were identified. Four of these showed high levels of homology with either murine Eck or Mek4 (7), but one clone (35C15) contained 1604 bp of sequence which appeared novel after comparative databank analysis. The novel molecule was termed embryonic stem cell kinase or Esk.

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A diagrammatic representation of Esk clones is shown in Figure 5 and the sequence of the Esk cDNA is shown in Figure 2.

EXAMPLE 11

5 ISOLATION OF A FULL-LENGTH Esk CLONE

The Esk cDNA was used to screen a mouse liver cDNA library and identified twenty-four positive clones. One clone containing the largest insert size was analysed in detail and found to include the complete coding region of Esk (Figure 2). This clone contained a single open
10 reading frame that encoded 977 amino acids, without an initiation codon occurring in a context consistent with the Kozac rules for translation start sites (42). Amino acids 1-26 conformed to the predicted sequence for a signal peptide (43). A sequence of predominantly hydrophobic residues at positions 549-569 were consistent with a transmembrane domain, potentially dividing the mature Esk protein into extracellular and intracellular portions. Within the
15 putative extracellular domain were 20 highly-conserved cysteine residues (indicated by circles in Figure 2) and two fibronectin type III repeats which are hallmarks of the Eph subfamily (42). In the putative intracellular domain, motifs typical of a protein-tyrosine kinase were seen (31). This included the GXGXXG ATP-binding motif (residues 632-637) and the associated invariant lysine at position 657, along with motifs indicating substrate specificity for tyrosine
20 (DLAARN, residues 750-755; and PIRWTAPE, residues 790-797). Finally, residues 60, 339, 415 and 479 are potential sites of N-linked glycosylation. Sequence database searching confirmed that the closest known Esk homologue was human Eph, with an overall amino acid sequence identity of 84.4%.

25 To confirm the localization of Esk, 135 grains over good-quality Chromosomes 6 were plotted onto the accurate idiogram of Evans (38). In this plot, the two tallest peaks of grains were again over bands 6B1 and 6B2 (Figure 6); together, these two peaks contained 56% of the total grains over Chromosome 6. The localization of the Esk gene to bands B1 and B2 of mouse Chromosome 6 was therefore confirmed by both sets of data.

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EXAMPLE 12**ANALYSIS OF THE RELATIONSHIP BETWEEN Esk AND Eph**

To determine whether Esk was the closest homologue of Eph in the mouse, a Southern blot
5 of mouse genomic DNA digested with different restriction enzymes was sequentially
hybridized to Esk and Eph probes. The probes were derived from exactly corresponding
regions of their respective coding sequence. As shown in Figure 7, both probes recognized
fragments of identical size with all restriction enzymes tested.

10

EXAMPLE 13**ESK EXPRESSION**

Northern analysis of Esk revealed expression of a 4.2 kb transcript in day-12 mouse embryo
and adult mouse thymus, liver, kidney, lung and placenta (Figure 3). Faint bands,
approximately 6.0 kb in size, were also observed in the liver, kidney and lung samples. This
15 may be due to alternatively spliced transcripts, although weak hybridization to related
molecules cannot be excluded. No signal was detected from lymph node, spleen, heart, brain
or skeletal muscle.

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EXAMPLE 14**COMPARATIVE EXPRESSION OF Esk, Mek4 AND Eck
IN THE ES CELL CULTURES**

To determine whether expression of Eph-subfamily molecules occurred in ES cells at a significant level and to investigate potential changes in expression as ES cells were differentiated *in vitro*, a Northern blot containing ES cell and embryoid body RNA was sequentially hybridized to Esk, Mek4 and Eck probes. Embryonic fibroblast RNA was included as a control on this blot, because some fibroblast contamination of the ES cell and embryoid body samples could not be excluded. As shown in Figure 4, the Esk probe hybridized to all three samples, approximately in proportion to the amount of RNA present. This result indicates significant levels of Esk expression in ES cells and embryoid bodies, and could not be accounted for by fibroblast contamination alone. In contrast, expression of Mek4 was barely detectable in the ES cell cultures. A 9.5 kb Mek4 transcript was expressed by the embryonic fibroblasts, however, suggesting that the faint bands seen in the ES cell lane may be due to contaminating fibroblast RNA. Finally, the Eck probe hybridized to all three samples, but expression was relatively greater in the undifferentiated ES cells. Transcripts were of slightly different sizes in the different lanes.

EXAMPLE 15**EXPRESSION**

In situ studies with digoxigenin-labelled ESK riboprobes were used to analyse expression using manufacturers protocols (Boehringer-Mannheim *In situ* hybridisation manual). Whole mount preparations of early (D8-12) mouse embryos show that ESK is expressed in the developing fore-gut and branchial arches. Later embryos were sectioned and analysed by *in situ* hybridisation. In these stages expression was noted in the thymus, basal layer of the skin, lung and in the developing eye. Similar studies were performed in tissue sections of adult tissues. In the adult animal expression was also seen in the thymus, as expected from Northern blot analysis, but was shown to be localised in the thymic epithelial cells. There was also detectable expression in the proximal and distal tubules of the kidney, the adrenal cortex and in testis. While expression was expected in other sites from the Northern analysis results, no

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signal greater than background was evident, perhaps suggesting low level expression of RNA degradation during processing.

Expression in epithelial but not in neural tissues confirms the impression that ESK is primarily involved in epithelial development. The expression in thymic epithelial cells is of particular interest as these cells have been shown to be involved in thymocyte selection and hence in development of T cell immune competence.

EXAMPLE 16

10 ANALYSIS OF Esk EXPRESSION BY *In situ* HYBRIDIZATION

The expression of Esk was investigated with *in situ* hybridization in embryoid bodies differentiated *in vitro*, mouse embryos and selected adult mouse tissues. These experiments were performed using two pairs of antisense and sense-control riboprobes derived from non-
15 overlapping regions of Esk sequence. Both antisense probes gave similar results. No specific staining was detected in any of the tissues hybridized to the sense-control probes with the exception of small intestine, where high levels of endogenous alkaline phosphatase activity resulted in false-positive staining of the epithelium. This occurred despite the inclusion of levamisole in the post-hybridization washes.

20

EXAMPLE 17

EMBRYOID BODIES

The strong expression of Esk in ES cells and embryoid bodies observed with Northern
25 analysis (Example 13) prompted investigation into whether expression was differentially regulated even at this early stage. In the current study *in situ* hybridization experiments were performed on embryoid bodies resulting from the differentiation of ES cells for 7-10 days *in vitro*. Differentiation was induced by growing the cells without a fibroblast feeder layer and in the absence of exogenous leukemia inhibitory factor (LIF), as previously
30 described (39). Specific binding of antisense probe, indicating Esk mRNA expression, was

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observed in both whole-mounted and sectioned embryoid bodies. In the whole-mounts, staining appeared strongest in the central region of embryoid bodies (Figure 8A). This distribution was confirmed by the sections. As shown in Figure 9A, although Esk was expressed by most embryoid-body cells, an amorphous layer visible near the surface at high magnification delineated an outer rind of cells that did not stain. The latter cells have been shown by others to correspond to primitive endoderm, whereas the cells within the interior are primitive ectoderm in type (45; 46). These populations of cells are separated by a layer of basement membrane-like material secreted by the endodermal cells.

10

EXAMPLE 18

EMBRYOS

To investigate Esk expression during later development, whole-mount preparations of embryos at days 8, 8.5, 9 and 9.5 of gestation were examined. The pattern of expression was similar in all embryos analysed and a representative specimen at day 9.5 of development is shown in Figure 8B. This demonstrates that Esk was predominantly expressed in ventral structures, including the branchial arches and the region containing the developing gut and its associated epithelial outgrowths. Expression was also observed in the region of the optic vesicle and there was lighter staining of the somites and other structures. No significant staining of the central nervous system was seen, which contrasts with the predominantly neural expression of many other Eph-subfamily receptors. Sections of day 18 embryos were also studied by *in situ* hybridization. These showed no specific staining for Esk mRNA in the majority of tissues. However, day 18 embryonic skin demonstrated positive staining in the deepest layers of the epidermis and in the developing hair follicles (Figure 9D). It is noteworthy that these are the regions of skin associated with active cellular proliferation and migration of cells into other layers.

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EXAMPLE 19

ADULT TISSUES

Northern analysis (Example 13) indicated that Esk is expressed in a variety of non-neural adult tissues. To define the specific cells containing Esk mRNA, *in situ* hybridisation experiments were performed on sections of some of these tissues. In thymus, Esk expression was observed in aggregates of large cells with abundant cytoplasm, located predominantly in the medulla (Figure 9B). Morphologically, these cells were consistent with thymic epithelia. No staining of thymocytes was observed. In kidney, Esk expression was localized to the epithelium of proximal and distal convoluted tubules (Figure 9C). Glomeruli and the interstitium did not stain. Esk was also expressed in the epithelial cells of the adrenal cortex. In sections of liver, small foci of hepatocytes stained positively, but the majority of the tissue was negative. As discussed earlier, no assessment of Esk expression in small intestine was possible, because of endogenous alkaline phosphatase activity.

15

EXAMPLE 20

CHROMOSOMAL LOCALIZATION

To determine the chromosomal localization of the Esk gene, a tritiated probe was made from the 1.6 kb cloned PCR fragment and hybridized *in situ* to preparations of mouse metaphase chromosomes. This resulted in a significant accumulation of silver grains over bands B1 and B2 of mouse Chromosome 6. Out of an initial score of 345 grains for all chromosomes, 36.8% of grains were over the proximal half of Chromosome 6. Background grains were distributed over the other chromosomes with 5 grains over distal Chromosome 15 to tallest secondary peak observed. This compared to 59 grains over band 6B2 and 28 over 6B1.

25

EXAMPLE 21

PREPARATION OF SECRETED RECOMBINANT Esk PROTEIN

30 1. MATERIALS AND METHODS

Cell lines

COS cells and CHO cells were propagated in RPMI containing 10% v/v heat-inactivated FCS in a humidified 10% v/v CO₂ atmosphere at 37°C. The COS cells were stably transfected with the polyoma large T antigen, permitting replication of vectors containing an SV40 origin of replication. For experiments involving selection of CHO cells cotransfected with the pSV2neo vector, a concentration of 600 µg/ml of G418 (Gibco BRL) in RPMI/1-% v/v FCS was used.

10 Assembly of an Esk-IgG Fc Construct

cDNA sequence was amplified from Esk using a 5' primer derived from sequence beginning five bases upstream of the translation start site and a 3' primer from sequence immediately upstream of the transmembrane domain. Custom *BglIII* sites were included in both primers and a splice donor sequence was added to the 3' primer. To minimize the possibility of PCR-induced errors, amplifications were performed using recombinant (Stratagene). The reaction was carried out in a 50 µl volume containing 1x cloned *Pfu* buffer, 0.2 mM each dNTP, 2.5 units cloned *Pfu* polymerase, 0.2 µM each primer and approximately 250 ng of pBluescript plasmid containing the full-length Esk clone, and was performed in a Perkin Elmer GeneAmp 2400 PCR machine. PCR amplification conditions were: 30 seconds at 95°C, 30 seconds at 55°C and 2 minutes and 30 seconds at 72°C for 5 cycles; 30 seconds at 95°C and 3 minutes at 72°C for 15 cycles. Amplification products were digested with *BglIII* and ligated into the unique *BamHI* cloning site of the plg-BOS expression vector. This plasmid is a pEF-BOS derivative which includes a genomic fragment of human IgG1 (containing exons encoding the hinge, CH2 and CH3 domains) downstream of the cloning site. Expression of this construct was expected to produce a polypeptide of approximately 90 kD in mass, which should become a divalent molecule of approximately 180 kD after the formation of cystine bonds between the hinge regions. The constructs were cloned in DH10 B *E.coli* cells and clones containing correctly oriented inserts were identified and DNA prepared and checked by DNA sequencing.

COS Cell Transfections

Five micrograms of expression plasmid prepared using a QIAGEN maxiprep kit was transiently transfected into COS cells using a standard DEAE-dextran method (35). A control
5 was also performed using identical conditions except no DNA was added. After three days of incubation, the culture supernatant was removed for analysis. Cells lysates were also obtained using 1% v/v Triton X-100 in 10mM Tris HCl (pH 7.4) and 150 mM NaCl.

CHO Cell Transfection and Subcloning

10

Ten micrograms of the Esk-IgG Fc expression construct and 1 μ g of pSV2neo G418-resistance plasmid were cotransfected into 2×10^7 CHO cells by electroporation. This was performed in 0.5 ml of PBS in a 4 mm sterile cuvette using 270 V and 960 μ FD on a BioRad Gene Pulser. Cells were incubated in 15 cm tissue culture dishes for 24 hours before selection with G418
15 was commenced. After the development of visible clones, these were subcloned into 24-well plates and the supernatant harvested for analysis when the cells were confluent.

Western Blots

20 Five microlitres of supernatant or cell lysate from the transfections described above was resolved by SDS polyacrylamid gel electrophoresis and transferred to a nitrocellulose membrane using established methods (35). Samples from the transfections were electrophoresed under both reducing and non-reducing conditions. Western blots were subsequently blocked in 5% w/v skimmed milk powder and 0.5% v/v Tween-20. After
25 blocking as both a rabbit anti-human Ig antibody (HRP-conjugated) and a rabbit anti-Esk peptide polyclonal serum (see below) the antibodies were used to probe the blots with a secondary layer consisting of an HRP-conjugated donkey anti-rabbit Ig antibody (Amersham) applied in the latter case. Detection was with the ECL kit (Amersham).

30 Production of An Anti-Esk peptide Polyclonal Serum

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A peptide was synthesized from the first 31 amino acids of the predicted mature protein encoded in the Esk sequence. This was coupled to keyhole limpet hemocyanin (KLH) and 200 μ g of the conjugate was emulsified in complete Freund's adjuvant and injected into a New Zealand white rabbit *via* multiple subcutaneous sites. A second dose of antigen was
5 administered in incomplete Freund's adjuvant after four weeks and serum was collected two weeks later. The serum was used at a dilution of 1:100 to probe Western blots.

Immunohistochemistry

10 CHO cells permanently transfected with an Esk and control CHO cells were grown in chamber slides, fixed in PBS containing 1%v/v paraformaldehyde for 30 minutes and permeabilized with 100% v/v methanol. After blocking in PBS containing 2% v/v FCS and 2% v/v goat serum, the cells were incubated with either a 1:100 dilution of the rabbit anti-Esk antiserum described in the previous section or with preimmune serum as a control. In a second set of
15 controls, 40 μ g of Esk peptide coupled to KLH was added with the antiserum. A donkey anti-rabbit Ig antibody conjugated to HRP was used as the secondary antibody in all cases and bound antibody was detected with diaminobenzidine (Dako).

Protein A affinity purification

The following method was used for protein A-Sepharose affinity chromatography. The column was pre-eluted with pH 3 0.1 M acetate buffer and re-equilibrated with PBS. The sample was applied and then the column washed to baseline with PBS. The sample was eluted with acetate buffer and peak fractions collected in tubes containing 1/10 vol of 1M tris HCl pH8. The column was re-equilibrated with PBS/azide for storage.

Biosensor studies

10

The studies were carried on a BIAcore 2000 (Pharmacia) using the manufacturers protocols. Individual channels of a biosensor CM chip were derivatised with ESK-Fc or with soluble HEK (47). Samples of LERK-2, -2, 03, -4, -5, -7 as Fc fusion proteins were analysed at 10 μ g/ml. Single chain forms of LERK-3 and -7 expressed as FLAGTM (Kodak) constructs were produced by the inventors and were analysed at 2 μ g/ml. In each case a parallel sample was run in the presence of excess ESK-Fc. The results are presented at the difference between these responses (ie. the ESK-specific component) for each test sample and are shown in Figure 10.

20 Polyclonal anti-Esk-Fc serum

The purified ESK Fc protein (100 μ g) was homogenised in complete Freund's adjuvant and administered subcutaneously at multiple sites. Animals were immunised every 14 days and bled 10 days after each injection. To purify the specific component the antiserum was exhaustively absorbed on a human immunoglobulin affinity column and then applied to an ESK Fc affinity column. the specific antibody was eluted with 0.2 M glycine HCl pH 2.3, quickly neutralised and concentrated to 0.5-1.0 mg/ml.

2. RESULTS

Analysis of the Rabbit Anti-Esk Polyclonal Serum

5 A polyclonal serum raised against an Esk peptide was initially tested by probing a Western blot containing recombinant Esk and Hek. The antibody recognized Esk specifically, with bands of the expected size appearing in both the full-length and truncated Esk samples and no specific bands in the Hek samples. When the anti-Esk serum was used for immunochemistry, the cytoplasm of CHO cells transfected with the Esk construct stained strongly. The
10 specificity of this staining for Esk was confirmed by several controls. These included incubations of Esk-transfected cells with preimmune serum and of control CHO cells with the antiserum. Only background staining was observed in either case. In addition, Esk peptide as observed to inhibit staining of the transfectants by the antiserum.

15 Transfection of the Esk-IgG Fc and CH48-IgG Fc Constructs

Western blots of the supernatants of COS cells transfected with the Esk-IgG construct. Specific bands of the expected size were detected in the transfected samples. Both the anti-Ig antibody and the anti-Esk antiserum gave positive signals. the Esk-Fc construct and a control
20 construct being a fusion between CD48 and IgG Fc were used to generate permanently-transfected CHO cells lines. Western blots of conditioned medium from the ESK-Fc clones revealed a similar pattern to that of the COS cell transfections. The CH48 Fc band was somewhat smaller. Lines which secreted very high levels of the fusion protein were selected.

25 Purification of ESK & CD48 Fc proteins

High level expressing clones were expanded to 1 litre cultures in RPMI 1640 supplemented with 2.5% v/v FCS which had been absorbed on protein A-Sepharose beads to remove all protein A-binding bovine immunoglobulin. The cultures were allowed to grow to confluence
30 and the conditioned medium removed, centrifuged to remove cellular material and

- 40 -

concentrated 10-fold. The medium was applied to a protein A Sepharose column as above. Purified protein was analysed by SDS-PAGE to confirm purity. In each case a single band was obtained on SDS-PAGE under reducing conditions. N terminal sequencing of the ESK-Fc protein band gave a single un-equivocal sequence:

5

GluGluValThrLeuMetAspThr [SEQ ID NO:9]

which matches residues 27-34 of the amino acid sequence of ESK derived from the nucleotide sequence.

10

Binding of LERK proteins to ESK Fc

The samples show the expected pattern of binding to soluble HEK. There is also clear evidence of binding of divalent LERK-1, -3 and -4 Fc samples to the Esk channel. However, 15 the predicted maximal binding would have given much larger responses (as seen on the HEK chip) suggesting that the binding is relatively weak. This is highlighted in the case of LERK-3 by the failure to detect any binding of monomeric ligand to Esk.

Those skilled in the art will appreciate that the invention described herein is susceptible to 20 variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 3
Eph-subfamily cDNA clones isolated from ES cells by RT-PCR

| Clone designation | Closest homologue | Homology (%) |
|---------------------------|-------------------|--------------|
| Primers P2 and P4* | | |
| 25C5 | Sek | 97.8 |
| 33C1.1 | Nuk | 97.2 |
| 33C1.2 | Eck | 99.1 |
| 33C1.5 | Eck | 100 |
| Primers P1 and P4* | | |
| 35C4 | Eck | 97.2 |
| 35C6 | Mek4 | 97.3 |
| 35C10 | Mek4 | 99.7 |
| 35C11 | Mek4 | 99.7 |
| 35C15 | Eph | 83 |

Homologues were identified by screening nucleic acid databanks with the FASTA program. Sek (11), Nuk (15), Eck (21) and Mek (8) are murine Eph-subfamily molecules; Eph (3) is a human molecule.

* Refers to the primers used in the initial amplification during RT-PCR.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: (OTHER THAN US) AMRAD OPERATIONS PTY LTD
(US ONLY):- Andrew Wallace BOYD and Jason Lickliter

(ii) TITLE OF INVENTION: A NOVEL RECEPTOR-TYPE TYROSINE KINASE
AND USE THEREOF

(iii) NUMBER OF SEQUENCES: 9

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3309 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..2931

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AACATATGTC CCCAAAGAGT TTAAAGCTGA GACCTTCACC TTCCACTCTG ATATCTGCAC      -175
ACTTCCAGAG AAGGAGAAGC AGATTAAGAA ACAAACGGCT CTGCTGAGC TGGTGAAGCA      -115
CAAGCCCAAG GCTACAGCGG AGCAACTGAA GACTGTCATG GATGACTTTG CACAGTTCCT      -55
GGATACATGT TGCAAGGCTG CTGGGAATTC CTGGGCCAGG TCCCGGCCGG CGCC              -1
ATG GAG CGG CGC TGG CCC CTG GGG CTT GCA TTG CTG CTG CTG CTG CTC          48
Met Glu Arg Arg Trp Pro Leu Gly Leu Ala Leu Leu Leu Leu Leu Leu
  1           5           10          15

TGC GCC CCG CTG CCC CCG GGG GCG CGC GCC GAG GAA GTC ACT CTA ATG          96
Cys Ala Pro Leu Pro Pro Gly Ala Arg Ala Glu Glu Val Thr Leu Met
          20           25           30

GAC ACA AGC ACA GCA CAA GGA GAG CTG GGC TGG CTT CTG GAT CCC CCA          144
Asp Thr Ser Thr Ala Gln Gly Glu Leu Gly Trp Leu Leu Asp Pro Pro
        35           40           45

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| | |
|---|-----|
| GAG ACT GGG TGG AGT GAG GTG CAA CAA ATG CTA AAC GGG ACA CCC CTG | 192 |
| Glu Thr Gly Trp Ser Glu Val Gln Gln Met Leu Asn Gly Thr Pro Leu | |
| 50 55 60 | |
| TAC ATG TAC CAA GAC TGC CCA ATA CAG GAA GGT GGG GAC ACT GAC CAC | 240 |
| Tyr Met Tyr Gln Asp Cys Pro Ile Gln Glu Gly Gly Asp Thr Asp His | |
| 65 70 75 80 | |
| TGG CTT CGC TCC AAT TGG ATC TAC CGC GGA GAG GAA GCT TCA CGC ATC | 288 |
| Trp Leu Arg Ser Asn Trp Ile Tyr Arg Gly Glu Glu Ala Ser Arg Ile | |
| 85 90 95 | |
| TAC GTG GAG CTG CAG TTC ACC GTG CGG GAC TGT AAG AGT TTC CCA GGG | 336 |
| Tyr Val Glu Leu Gln Phe Thr Val Arg Asp Cys Lys Ser Phe Pro Gly | |
| 100 105 110 | |
| GGA GCT GGG CCT CTG GGA TGC AAA GAG ACC TTC AAC CTT TTC TAC ATG | 384 |
| Gly Ala Gly Pro Leu Gly Cys Lys Glu Thr Phe Asn Leu Phe Tyr Met | |
| 115 120 125 | |
| GAG AGT GAC CAG GAT GTG GGC ATT CAG CTC CGA CGA CCT TTG TTC CAA | 432 |
| Glu Ser Asp Gln Asp Val Gly Ile Gln Leu Arg Arg Pro Leu Phe Gln | |
| 130 135 140 | |
| AAG GTA ACA ACT GTG GCA GCA GAC CAG AGC TTC ACC ATC AGA GAC CTG | 480 |
| Lys Val Thr Thr Val Ala Ala Asp Gln Ser Phe Thr Ile Arg Asp Leu | |
| 145 150 155 160 | |
| GCA TCT GAC TCT GTA AAG CTG AAT GTA GAA CGC TGC TCG TTG GGC CAC | 528 |
| Ala Ser Asp Ser Val Lys Leu Asn Val Glu Arg Cys Ser Leu Gly His | |
| 165 170 175 | |
| CTC ACC CGC CGT GGC CTC TAC TTA GCT TTC CAC AAC CCG GGT TCC TGT | 576 |
| Leu Thr Arg Arg Gly Leu Tyr Leu Ala Phe His Asn Pro Gly Ser Cys | |
| 180 185 190 | |
| GTG GCG CTA GTG TCT GTA AGG GTG TTC TAC CAG CGC TGT GCC GAG ACC | 624 |
| Val Ala Leu Val Ser Val Arg Val Phe Tyr Gln Arg Cys Ala Glu Thr | |
| 195 200 205 | |
| GTG CAT GGC TTG GCC CAC TTC CCT GAC ACT CTC CCT GGA CCT GGA GGG | 672 |
| Val His Gly Leu Ala His Phe Pro Asp Thr Leu Pro Gly Pro Gly Gly | |
| 210 215 220 | |
| TTG GTT GAA GTA GCT GGA ACG TGC CTC TCC CAT GCA CAG ATC AGC TTG | 720 |
| Leu Val Glu Val Ala Gly Thr Cys Leu Ser His Ala Gln Ile Ser Leu | |

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| | | | | |
|---|-----|-----|-----|------|
| 225 | 230 | 235 | 240 | |
| GGG TCC TCA GGT ACA CCA CGA ATG CAC TGC AGC CCT GAT GGC GAG TGG | | | | 768 |
| Gly Ser Ser Gly Thr Pro Arg Met His Cys Ser Pro Asp Gly Glu Trp | | | | |
| | 245 | 250 | 255 | |
| CTG GTG CCT GTG GGT CAG TGC CAG TGC GAG CCT GGC TAT GAA GAA AGC | | | | 816 |
| Leu Val Pro Val Gly Gln Cys Gln Cys Glu Pro Gly Tyr Glu Glu Ser | | | | |
| | 260 | 265 | 270 | |
| AGT GGA AAT GTG GGA TGC ACT GCC TGT CCT ACT GGT TTC TAT CGA GTG | | | | 864 |
| Ser Gly Asn Val Gly Cys Thr Ala Cys Pro Thr Gly Phe Tyr Arg Val | | | | |
| | 275 | 280 | 285 | |
| GAC ATG AAT ACA CTC CGT TGT CTC AAG TGC CCC CAA CAT AGC ATA GCA | | | | 912 |
| Asp Met Asn Thr Leu Arg Cys Leu Lys Cys Pro Gln His Ser Ile Ala | | | | |
| | 290 | 295 | 300 | |
| GAG TCT GAG GGG TCT ACC ATC TGT ACC TGT GAG AAT GGA CAT TAT CGA | | | | 960 |
| Glu Ser Glu Gly Ser Thr Ile Cys Thr Cys Glu Asn Gly His Tyr Arg | | | | |
| | 305 | 310 | 315 | 320 |
| GCC CCT GGG GAG GGT CCC CAG GTA GCA TGC ACA CGT CCC CCA TCG GCT | | | | 1008 |
| Ala Pro Gly Glu Gly Pro Gln Val Ala Cys Thr Arg Pro Pro Ser Ala | | | | |
| | 325 | 330 | 335 | |
| CCC CAA AAT CTG AGC TTC TCC ACA TCA GGG ACT CAA CTC TCC CTG CGC | | | | 1056 |
| Pro Gln Asn Leu Ser Phe Ser Thr Ser Gly Thr Gln Leu Ser Leu Arg | | | | |
| | 340 | 345 | 350 | |
| TGG GAG CCC CCC AGA GAT ACA GGG GGA CGC CAT GAT ATC AGA TAC AGC | | | | 1104 |
| Trp Glu Pro Pro Arg Asp Thr Gly Gly Arg His Asp Ile Arg Tyr Ser | | | | |
| | 355 | 360 | 365 | |
| GTG GAG TGC TTG CAG TGT CGG GGC ATT GCA CAG GAT GGG GGT CCC TGC | | | | 1152 |
| Val Glu Cys Leu Gln Cys Arg Gly Ile Ala Gln Asp Gly Gly Pro Cys | | | | |
| | 370 | 375 | 380 | |
| CAA CCC TGT GGA AAA GGT GTG CAC TTT TCC CCG GCT GCT TCC GGG CTC | | | | 1200 |
| Gln Pro Cys Gly Lys Gly Val His Phe Ser Pro Ala Ala Ser Gly Leu | | | | |
| | 385 | 390 | 395 | 400 |
| ACC ACA TCT ACC GTG CAA GTG CAA GGC CTC GAG CCT TAC GCC AAC TAC | | | | 1248 |
| Thr Thr Ser Thr Val Gln Val Gln Gly Leu Glu Pro Tyr Ala Asn Tyr | | | | |
| | 405 | 410 | 415 | |

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| | |
|---|------|
| ACA TTT ACC GTC AAA TCC CAA AAC AGA GTG TCA GGA CTG GAC AGT TCC | 1296 |
| Thr Phe Thr Val Lys Ser Gln Asn Arg Val Ser Gly Leu Asp Ser Ser | |
| 420 425 430 | |
| AGC CCT AGC AGC GCC TCC CTC AGT ATC AAC ATG GGG CAC GCA GAG TCA | 1344 |
| Ser Pro Ser Ser Ala Ser Leu Ser Ile Asn Met Gly His Ala Glu Ser | |
| 435 440 445 | |
| CTC TCT GGC CTG TCA CTG AAG CTG GTG AAG AAA GAA CCG AGG CAG CTG | 1392 |
| Leu Ser Gly Leu Ser Leu Lys Leu Val Lys Lys Glu Pro Arg Gln Leu | |
| 450 455 460 | |
| GAG CTG ACT TGG GCA GGG TCC CGA CCC CGA AAT CCT GGA GGG AAT CTG | 1440 |
| Glu Leu Thr Trp Ala Gly Ser Arg Pro Arg Asn Pro Gly Gly Asn Leu | |
| 465 470 475 480 | |
| AGC TAT GAG CTG CAC GTG CTG AAT CAG GAC GAA GAA TGG CAC CAG ATG | 1488 |
| Ser Tyr Glu Leu His Val Leu Asn Gln Asp Glu Glu Trp His Gln Met | |
| 485 490 495 | |
| GTG TTG GAA CCC AGG GTC TTG CTG ACA AAA CTT CAG CCA GAT ACC ACA | 1536 |
| Val Leu Glu Pro Arg Val Leu Leu Thr Lys Leu Gln Pro Asp Thr Thr | |
| 500 505 510 | |
| TAC ATT GTC AGA GTG CGA ACA CTG ACC CCA CTG GGG CCT GGC CCT TTC | 1584 |
| Tyr Ile Val Arg Val Arg Thr Leu Thr Pro Leu Gly Pro Gly Pro Phe | |
| 515 520 525 | |
| TCC CCT GAC CAT GAG TTT CGG ACA AGC CCA CCA GTT TCC AGA AGC CTG | 1632 |
| Ser Pro Asp His Glu Phe Arg Thr Ser Pro Pro Val Ser Arg Ser Leu | |
| 530 535 540 | |
| ACC GGA GGA GAG ATT GTG GCC GTC ATC TTT GGA TTG CTG CTT GGA ATA | 1680 |
| Thr Gly Gly Glu Ile Val Ala Val Ile Phe Gly Leu Leu Leu Gly Ile | |
| 545 550 555 560 | |
| GCT CTG CTG ATC GGG ATT TAT GTC TTC CGT TCA AGG AGA GGC CAG AGA | 1728 |
| Ala Leu Leu Ile Gly Ile Tyr Val Phe Arg Ser Arg Arg Gly Gln Arg | |
| 565 570 575 | |
| CAG AGA CAG CAG AGG CAG CGT GAA CGC ACC ACC AAT GTC GAT CGA GAG | 1776 |
| Gln Arg Gln Gln Arg Gln Arg Glu Arg Thr Thr Asn Val Asp Arg Glu | |
| 580 585 590 | |
| GAC AAG CTG TGG CTA AAA CCC TAT GTG GAC CTC CAG GCC TAT GAG GAC | 1824 |
| Asp Lys Leu Trp Leu Lys Pro Tyr Val Asp Leu Gln Ala Tyr Glu Asp | |

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| 595 | 600 | 605 | |
|---|-----|-----|------|
| CCT GCA CAG GGA GCC TTA GAC TTT GCC CAG GAA CTG GAC CCA GCC TGG | | | 1872 |
| Pro Ala Gln Gly Ala Leu Asp Phe Ala Gln Glu Leu Asp Pro Ala Trp | | | |
| 610 | 615 | 620 | |
| CTG ATT GTG GAC ACT GTC ATA GGA GAA GGG GAG TTT GGT GAA GTG TAT | | | 1920 |
| Leu Ile Val Asp Thr Val Ile Gly Glu Gly Glu Phe Gly Glu Val Tyr | | | |
| 625 | 630 | 635 | 640 |
| CGG GGA GCC CTG AGA CTC CCC AGC CAA GAT TGC AAG ACT GTG GCC ATT | | | 1968 |
| Arg Gly Ala Leu Arg Leu Pro Ser Gln Asp Cys Lys Thr Val Ala Ile | | | |
| 645 | 650 | 655 | |
| AAG ACC TTG AAA GAT ACA TCC CCA GAT GGC TAC TGG TGG AAT TTC CTT | | | 2016 |
| Lys Thr Leu Lys Asp Thr Ser Pro Asp Gly Tyr Trp Trp Asn Phe Leu | | | |
| 660 | 665 | 670 | |
| CGA GAG GCA ACT ATC ATG GGC CAG TTC AAC CAC CCA CAC ATT CTA CGC | | | 2064 |
| Arg Glu Ala Thr Ile Met Gly Gln Phe Asn His Pro His Ile Leu Arg | | | |
| 675 | 680 | 685 | |
| CTA GAA GGT GTC ATC ACA AAA AGA AAG CCC ATC ATG ATC ATC ACA GAA | | | 2112 |
| Leu Glu Gly Val Ile Thr Lys Arg Lys Pro Ile Met Ile Ile Thr Glu | | | |
| 690 | 695 | 700 | |
| TTT ATG GAA AAT GGA GCC CTG GAT GCC TTT CTG AAG GAA CGG GAG GAC | | | 2160 |
| Phe Met Glu Asn Gly Ala Leu Asp Ala Phe Leu Lys Glu Arg Glu Asp | | | |
| 705 | 710 | 715 | 720 |
| CAA CTA GCT CCT GGT CAG CTA GTG GCT ATG CTA CTG GGC ATA GCA TCA | | | 2208 |
| Gln Leu Ala Pro Gly Gln Leu Val Ala Met Leu Leu Gly Ile Ala Ser | | | |
| 725 | 730 | 735 | |
| GGC ATG AAC TGC CTC AGT GGC CAC AAT TAT GTC CAT AGA GAC CTG GCT | | | 2256 |
| Gly Met Asn Cys Leu Ser Gly His Asn Tyr Val His Arg Asp Leu Ala | | | |
| 740 | 745 | 750 | |
| GCC AGG AAC ATC TTG GTG AAT CAG AAC CTG TGC TGC AAG GTG TCT GAC | | | 2304 |
| Ala Arg Asn Ile Leu Val Asn Gln Asn Leu Cys Cys Lys Val Ser Asp | | | |
| 755 | 760 | 765 | |
| TTT GGC TTG ACC CGC CTC CTG GAT GAC TTT GAC GGC ACC TAT GAA ACC | | | 2352 |
| Phe Gly Leu Thr Arg Leu Leu Asp Asp Phe Asp Gly Thr Tyr Glu Thr | | | |
| 770 | 775 | 780 | |

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| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| CAG | GGA | GGG | AAG | ATC | CCC | ATC | CGA | TGG | ACA | GCC | CCA | GAA | GCT | ATT | GCC | 2400 |
| Gln | Gly | Gly | Lys | Ile | Pro | Ile | Arg | Trp | Thr | Ala | Pro | Glu | Ala | Ile | Ala | |
| 785 | | | | | 790 | | | | | 795 | | | | | 800 | |
| CAT | CGG | ATC | TTC | ACC | ACA | GCC | AGT | GAT | GTG | TGG | AGC | TTT | GGG | ATT | GTA | 2448 |
| His | Arg | Ile | Phe | Thr | Thr | Ala | Ser | Asp | Val | Trp | Ser | Phe | Gly | Ile | Val | |
| | | | | 805 | | | | | 810 | | | | | 815 | | |
| ACG | TGG | GAG | GTG | TTG | AGT | TTT | GGC | GAC | AAA | CCC | TAT | GGG | GAG | ATG | AGC | 2496 |
| Thr | Trp | Glu | Val | Leu | Ser | Phe | Gly | Asp | Lys | Pro | Tyr | Gly | Glu | Met | Ser | |
| | | | 820 | | | | | 825 | | | | | 830 | | | |
| AAC | CAA | GAG | GTA | ATG | AAA | AGC | ATT | GAA | GAT | GGG | TAC | CGG | TTG | CCC | CCT | 2544 |
| Asn | Gln | Glu | Val | Met | Lys | Ser | Ile | Glu | Asp | Gly | Tyr | Arg | Leu | Pro | Pro | |
| | | 835 | | | | | 840 | | | | | 845 | | | | |
| CCT | GTG | GAC | TGT | CCT | GCC | CCT | CTC | TAT | GAA | CTC | ATG | AAG | AAC | TGC | TGG | 2592 |
| Pro | Val | Asp | Cys | Pro | Ala | Pro | Leu | Tyr | Glu | Leu | Met | Lys | Asn | Cys | Trp | |
| | 850 | | | | | 855 | | | | | 860 | | | | | |
| GCT | TAC | GAT | CGT | GCC | CGT | CGA | CCC | CAC | TTC | CTC | CAG | CTG | CAG | GCA | CAT | 2640 |
| Ala | Tyr | Asp | Arg | Ala | Arg | Arg | Pro | His | Phe | Leu | Gln | Leu | Gln | Ala | His | |
| 865 | | | | | 870 | | | | | 875 | | | | | 880 | |
| CTG | GAA | CAG | TTG | CTT | ACT | GAC | CCC | CAT | TCC | CTA | AGG | ACA | ATT | GCC | AAC | 2688 |
| Leu | Glu | Gln | Leu | Leu | Thr | Asp | Pro | His | Ser | Leu | Arg | Thr | Ile | Ala | Asn | |
| | | | 885 | | | | | | 890 | | | | | 895 | | |
| TTT | GAC | CCT | AGG | GTG | ACC | TTA | CGC | CTG | CCC | AGC | CTG | AGT | GGC | TCT | GAT | 2736 |
| Phe | Asp | Pro | Arg | Val | Thr | Leu | Arg | Leu | Pro | Ser | Leu | Ser | Gly | Ser | Asp | |
| | | 900 | | | | | | 905 | | | | | 910 | | | |
| GGG | ATC | CCT | TAT | CGA | AGT | GTC | TCT | GAG | TGG | CTT | GAA | TCC | ATA | CGC | ATG | 2784 |
| Gly | Ile | Pro | Tyr | Arg | Ser | Val | Ser | Glu | Trp | Leu | Glu | Ser | Ile | Arg | Met | |
| | 915 | | | | | | 920 | | | | | 925 | | | | |
| AAG | CGC | TAC | ATC | CTG | CAC | TTC | CGT | TCG | GCT | GGG | CTG | GAC | ACC | ATG | GAG | 2832 |
| Lys | Arg | Tyr | Ile | Leu | His | Phe | Arg | Ser | Ala | Gly | Leu | Asp | Thr | Met | Glu | |
| | 930 | | | | | 935 | | | | | 940 | | | | | |
| TGT | GTG | CTG | GAG | CTG | ACG | GCT | GAG | GAC | CTG | ACG | CAG | ATG | GGA | ATA | ACG | 2880 |
| Cys | Val | Leu | Glu | Leu | Thr | Ala | Glu | Asp | Leu | Thr | Gln | Met | Gly | Ile | Thr | |
| 945 | | | | | 950 | | | | | 955 | | | | | 960 | |
| TTG | CCA | GGG | CAC | CAG | AAA | CGA | ATT | CTC | TGC | AGT | ATT | CAA | GGA | TTT | AAG | 2928 |
| Leu | Pro | Gly | His | Gln | Lys | Arg | Ile | Leu | Cys | Ser | Ile | Gln | Gly | Phe | Lys | |

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| 965 | 970 | 975 | |
|--|-----|-----|------|
| GAC TGAGCATCCA CTGAAAAGAT GCTCCAGCCC TCTGCCTGCC TCCATTAGCA | | | 2981 |
| Aap | | | |
| AGGACGGGGT ACAGTCAACT CCCTGGGCCT TTCCTCAGCC TACGAAATGT AGGCTATTGG | | | 3041 |
| TGCTGCTCCT GCCCAGTCAA TCAGAACTCT GCCTTTGAAC CAAGGAGCCT TTGTTTATAA | | | 3101 |
| AGGGGGTGGA TGGGTACAAG TGAAGGGGnC TGTGGGTGGG TTCTGGGGGA GGGTTTAATA | | | 3161 |
| nATACACTTA CATATGCATT ATCTATTTTT GTAAATAAAC AAGAGTTGAG TTTTAAAAAA | | | 3221 |
| AAAAAAAAAAG GAATTCCTGC AGCCCGGGGG ATCCACTAGT TCTAGAGCGG CCGCCACCGC | | | 3281 |
| GGTGGAGCTC CAGCTTTTGT TCCCTTTA | | | 3309 |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 977 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Glu | Arg | Arg | Trp | Pro | Leu | Gly | Leu | Ala | Leu | Leu | Leu | Leu | Leu | Leu |
| 1 | | | | | 5 | | | | | 10 | | | | | 15 |
| Cys | Ala | Pro | Leu | Pro | Pro | Gly | Ala | Arg | Ala | Glu | Glu | Val | Thr | Leu | Met |
| | | | 20 | | | | | 25 | | | | | | 30 | |
| Asp | Thr | Ser | Thr | Ala | Gln | Gly | Glu | Leu | Gly | Trp | Leu | Leu | Asp | Pro | Pro |
| | | | 35 | | | | 40 | | | | | | 45 | | |
| Glu | Thr | Gly | Trp | Ser | Glu | Val | Gln | Gln | Met | Leu | Asn | Gly | Thr | Pro | Leu |
| | | | 50 | | | | 55 | | | | | 60 | | | |
| Tyr | Met | Tyr | Gln | Asp | Cys | Pro | Ile | Gln | Glu | Gly | Gly | Asp | Thr | Asp | His |
| | | | 65 | | | 70 | | | | | 75 | | | | 80 |
| Trp | Leu | Arg | Ser | Asn | Trp | Ile | Tyr | Arg | Gly | Glu | Glu | Ala | Ser | Arg | Ile |

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| | | | | | |
|---|-----|-----|-----|-----|-----|
| | 85 | | 90 | | 95 |
| Tyr Val Glu Leu Gln Phe Thr Val Arg Asp Cys Lys Ser Phe Pro Gly | | | | | |
| | 100 | | 105 | | 110 |
| Gly Ala Gly Pro Leu Gly Cys Lys Glu Thr Phe Asn Leu Phe Tyr Met | | | | | |
| | 115 | | 120 | | 125 |
| Glu Ser Asp Gln Asp Val Gly Ile Gln Leu Arg Arg Pro Leu Phe Gln | | | | | |
| | 130 | | 135 | | 140 |
| Lys Val Thr Thr Val Ala Ala Asp Gln Ser Phe Thr Ile Arg Asp Leu | | | | | |
| | 145 | | 150 | | 155 |
| Ala Ser Asp Ser Val Lys Leu Asn Val Glu Arg Cys Ser Leu Gly His | | | | | |
| | | 165 | | 170 | 175 |
| Leu Thr Arg Arg Gly Leu Tyr Leu Ala Phe His Asn Pro Gly Ser Cys | | | | | |
| | | 180 | | 185 | 190 |
| Val Ala Leu Val Ser Val Arg Val Phe Tyr Gln Arg Cys Ala Glu Thr | | | | | |
| | 195 | | 200 | | 205 |
| Val His Gly Leu Ala His Phe Pro Asp Thr Leu Pro Gly Pro Gly Gly | | | | | |
| | 210 | | 215 | | 220 |
| Leu Val Glu Val Ala Gly Thr Cys Leu Ser His Ala Gln Ile Ser Leu | | | | | |
| | 225 | | 230 | | 235 |
| Gly Ser Ser Gly Thr Pro Arg Met His Cys Ser Pro Asp Gly Glu Trp | | | | | |
| | | 245 | | 250 | 255 |
| Leu Val Pro Val Gly Gln Cys Gln Cys Glu Pro Gly Tyr Glu Glu Ser | | | | | |
| | 260 | | 265 | | 270 |
| Ser Gly Asn Val Gly Cys Thr Ala Cys Pro Thr Gly Phe Tyr Arg Val | | | | | |
| | 275 | | 280 | | 285 |
| Asp Met Asn Thr Leu Arg Cys Leu Lys Cys Pro Gln His Ser Ile Ala | | | | | |
| | 290 | | 295 | | 300 |
| Glu Ser Glu Gly Ser Thr Ile Cys Thr Cys Glu Asn Gly His Tyr Arg | | | | | |
| | 305 | | 310 | | 315 |
| Ala Pro Gly Glu Gly Pro Gln Val Ala Cys Thr Arg Pro Pro Ser Ala | | | | | |
| | 325 | | 330 | | 335 |

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Pro Gln Asn Leu Ser Phe Ser Thr Ser Gly Thr Gln Leu Ser Leu Arg
 340 345 350

Trp Glu Pro Pro Arg Asp Thr Gly Gly Arg His Asp Ile Arg Tyr Ser
 355 360 365

Val Glu Cys Leu Gln Cys Arg Gly Ile Ala Gln Asp Gly Gly Pro Cys
 370 375 380

Gln Pro Cys Gly Lys Gly Val His Phe Ser Pro Ala Ala Ser Gly Leu
 385 390 395 400

Thr Thr Ser Thr Val Gln Val Gln Gly Leu Glu Pro Tyr Ala Asn Tyr
 405 410 415

Thr Phe Thr Val Lys Ser Gln Asn Arg Val Ser Gly Leu Asp Ser Ser
 420 425 430

Ser Pro Ser Ser Ala Ser Leu Ser Ile Asn Met Gly His Ala Glu Ser
 435 440 445

Leu Ser Gly Leu Ser Leu Lys Leu Val Lys Lys Glu Pro Arg Gln Leu
 450 455 460

Glu Leu Thr Trp Ala Gly Ser Arg Pro Arg Asn Pro Gly Gly Asn Leu
 465 470 475 480

Ser Tyr Glu Leu His Val Leu Asn Gln Asp Glu Glu Trp His Gln Met
 485 490 495

Val Leu Glu Pro Arg Val Leu Leu Thr Lys Leu Gln Pro Asp Thr Thr
 500 505 510

Tyr Ile Val Arg Val Arg Thr Leu Thr Pro Leu Gly Pro Gly Pro Phe
 515 520 525

Ser Pro Asp His Glu Phe Arg Thr Ser Pro Pro Val Ser Arg Ser Leu
 530 535 540

Thr Gly Gly Glu Ile Val Ala Val Ile Phe Gly Leu Leu Leu Gly Ile
 545 550 555 560

Ala Leu Leu Ile Gly Ile Tyr Val Phe Arg Ser Arg Arg Gly Gln Arg
 565 570 575

Gln Arg Gln Gln Arg Gln Arg Glu Arg Thr Thr Asn Val Asp Arg Glu

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| | | |
|---|-----|---------|
| 580 | 585 | 590 |
| Asp Lys Leu Trp Leu Lys Pro Tyr Val Asp Leu Gln Ala Tyr Glu Asp | | |
| 595 | 600 | 605 |
| Pro Ala Gln Gly Ala Leu Asp Phe Ala Gln Glu Leu Asp Pro Ala Trp | | |
| 610 | 615 | 620 |
| Leu Ile Val Asp Thr Val Ile Gly Glu Gly Glu Phe Gly Glu Val Tyr | | |
| 625 | 630 | 635 640 |
| Arg Gly Ala Leu Arg Leu Pro Ser Gln Asp Cys Lys Thr Val Ala Ile | | |
| 645 | 650 | 655 |
| Lys Thr Leu Lys Asp Thr Ser Pro Asp Gly Tyr Trp Trp Asn Phe Leu | | |
| 660 | 665 | 670 |
| Arg Glu Ala Thr Ile Met Gly Gln Phe Asn His Pro His Ile Leu Arg | | |
| 675 | 680 | 685 |
| Leu Glu Gly Val Ile Thr Lys Arg Lys Pro Ile Met Ile Ile Thr Glu | | |
| 690 | 695 | 700 |
| Phe Met Glu Asn Gly Ala Leu Asp Ala Phe Leu Lys Glu Arg Glu Asp | | |
| 705 | 710 | 715 720 |
| Gln Leu Ala Pro Gly Gln Leu Val Ala Met Leu Leu Gly Ile Ala Ser | | |
| 725 | 730 | 735 |
| Gly Met Asn Cys Leu Ser Gly His Asn Tyr Val His Arg Asp Leu Ala | | |
| 740 | 745 | 750 |
| Ala Arg Asn Ile Leu Val Asn Gln Asn Leu Cys Cys Lys Val Ser Asp | | |
| 755 | 760 | 765 |
| Phe Gly Leu Thr Arg Leu Leu Asp Asp Phe Asp Gly Thr Tyr Glu Thr | | |
| 770 | 775 | 780 |
| Gln Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ala | | |
| 785 | 790 | 795 800 |
| His Arg Ile Phe Thr Thr Ala Ser Asp Val Trp Ser Phe Gly Ile Val | | |
| 805 | 810 | 815 |
| Thr Trp Glu Val Leu Ser Phe Gly Asp Lys Pro Tyr Gly Glu Met Ser | | |
| 820 | 825 | 830 |

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Asn Gln Glu Val Met Lys Ser Ile Glu Asp Gly Tyr Arg Leu Pro Pro
 835 840 845

Pro Val Asp Cys Pro Ala Pro Leu Tyr Glu Leu Met Lys Asn Cys Trp
 850 855 860

Ala Tyr Asp Arg Ala Arg Arg Pro His Phe Leu Gln Leu Gln Ala His
 865 870 875 880

Leu Glu Gln Leu Leu Thr Asp Pro His Ser Leu Arg Thr Ile Ala Asn
 885 890 895

Phe Asp Pro Arg Val Thr Leu Arg Leu Pro Ser Leu Ser Gly Ser Asp
 900 905 910

Gly Ile Pro Tyr Arg Ser Val Ser Glu Trp Leu Glu Ser Ile Arg Met
 915 920 925

Lys Arg Tyr Ile Leu His Phe Arg Ser Ala Gly Leu Asp Thr Met Glu
 930 935 940

Cys Val Leu Glu Leu Thr Ala Glu Asp Leu Thr Gln Met Gly Ile Thr
 945 950 955 960

Leu Pro Gly His Gln Lys Arg Ile Leu Cys Ser Ile Gln Gly Phe Lys
 965 970 975

Asp

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTAGGCATGC AAGGAGAC(A/C) TT(C/T)AACC

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGATGATCA T(C/G)AC(A/G/T)GA(A/G)TA (C/T)ATGG

25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTAGGAATTC CA(C/G/T)ACATC(A/G)C T(A/G)GC

24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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CCA(T/A)A(A/G)CTCC A(C/T)ACATC(A/G)CT

20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Leu Ala Ala Arg Asn

5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Ile Arg Trp Thr Ala Pro

5

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(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Glu Glu Val Thr Leu Met Asp Thr

5

CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a receptor tyrosine kinase (RTK) or a derivative, homologue or chemical analogue thereof, said RTK having the following characteristics:
 - (i) belongs to the Eph subfamily of RTKs as determined by conserved cysteine residues and fibronectin type III repeats;
 - (ii) comprises protein tyrosine kinase catalytic domain motifs; and
 - (iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79% similarity thereto.
2. An isolated nucleic acid molecule according to claim 1 wherein the RTK is of mammalian origin.
3. An isolated nucleic acid molecule according to claim 2 wherein the RTK is of human or murine origin.
4. An isolated nucleic acid molecule according to claim 1 comprising a nucleotide sequence substantially as set forth in SEQ ID NO: 1 or having at least about 82% similarity thereto or is capable of hybridizing to the sequence set forth in SEQ ID NO: 1 or a complementary form thereof under low stringency conditions.
5. An isolated nucleic acid molecule according to claim 1 or 2 or 3 or 4 contained in a vector.
6. An isolated nucleic acid molecule according to claim 5 wherein the vector is an expression vector.
7. An isolated nucleic acid molecule according to claim 1 or 4 encoding a soluble form of said RTK.

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8. A recombinant polypeptide or a derivative, homologue or chemical analogue thereof having the following characteristics:
 - (i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;
 - (ii) comprises protein tyrosine kinase catalytic domain motifs; and
 - (iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO:2 or having at least about 79% similarity thereto.
9. A recombinant polypeptide according to claim 8 wherein said RTK is of mammalian origin.
10. A recombinant polypeptide according to claim 9 wherein the RTK is of human or murine origin.
11. A recombinant polypeptide according to claim 8 encoded by a nucleotide sequence substantially as set forth in SEQ ID NO: 1 or having at least about 82% similarity thereto or a nucleotide sequence capable of hybridizing to the sequence set forth in SEQ ID NO: 1 or a complementary form thereof under low stringency conditions.
12. A recombinant polypeptide according to any one of claims 8 to 11 in soluble form.
13. A secreted recombinant polypeptide comprising conserved cysteine residues and fibronectin type III repeats and an extracellular portion of the amino acid sequence set forth in SEQ ID NO:2 or having at least about 79% similarity thereto.
14. A pharmaceutical composition comprising a recombinant polypeptide according to any one of claims 8 to 13 and one or more pharmaceutically acceptable carriers and/or diluents.
15. A method for modulating Esk-ligand interaction in an animal, said method comprising administering to said animal a modulating effective amount of an agonist or antagonist of Esk-

ligand interaction.

16. A method according to claim 15 wherein the Esk has the following characteristics:
 - (i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;
 - (ii) comprises protein tyrosine kinase catalytic domain motifs; and
 - (iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79% similarity thereto.
17. An antagonist or agonist of an Esk, said Esk having the following characteristics:
 - (i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;
 - (ii) comprises protein tyrosine kinase catalytic domain motifs; and
 - (iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79% similarity thereto.
18. An antagonist according to claim 17 wherein said antagonist is an antibody to said Esk.
19. An antibody to the recombinant polypeptide of claim 8.
20. An antibody to the secreted recombinant polypeptide of claim 13.

1/30

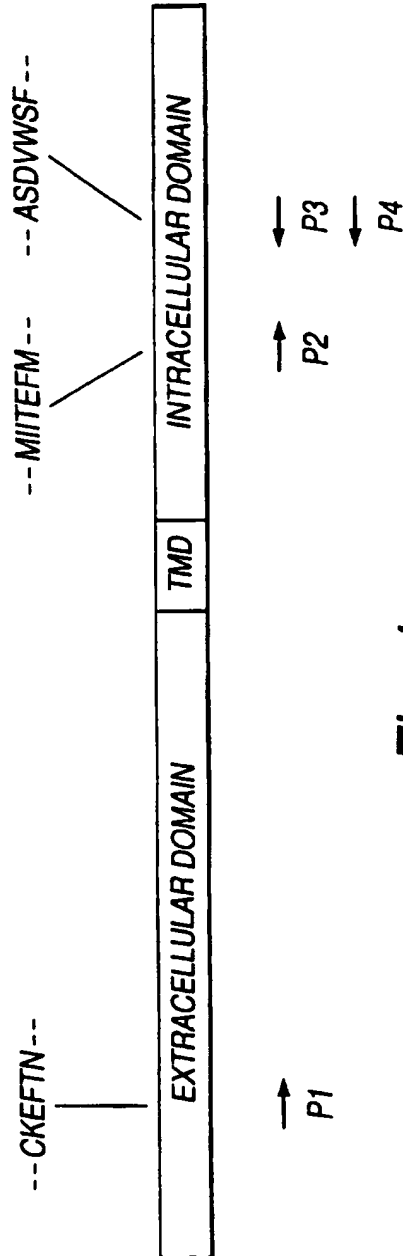


Fig. 1

2/30

| | |
|--------------|--------------|
| <i>3/30</i> | <i>4/30</i> |
| <i>5/30</i> | <i>6/30</i> |
| <i>7/30</i> | <i>8/30</i> |
| <i>9/30</i> | <i>10/30</i> |
| <i>11/30</i> | <i>12/30</i> |
| <i>13/30</i> | <i>14/30</i> |
| <i>15/30</i> | <i>16/30</i> |
| <i>17/30</i> | <i>18/30</i> |
| <i>19/30</i> | <i>20/30</i> |
| <i>21/30</i> | <i>22/30</i> |

Fig. 2

3/30

Tatggtgaac cgtgtgtgtc tgctgcatga

Gtgctgtagt ggatccctgg tggaaaggcg

Aacatatgtc cccaaagagt ttaaagctga

Acttccagag aaggagaagc agattaagaa

Caagcccaag gctacagcgg agcaactgaa

Ggatacatgt tgcaaggctg ctgggaattc

ATG GAG CGG CGC TGG CCC CTG GGG

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Glu | Arg | Arg | Trp | Pro | Leu | Gly |
|-----|-----|-----|-----|-----|-----|-----|-----|

TGC GCC CCG CTG CCC CCG GGG GCG

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Ala | Pro | Leu | Pro | Pro | Gly | Ala |
|-----|-----|-----|-----|-----|-----|-----|-----|

GAC ACA AGC ACA GCA CAA GGA GAG

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Thr | Ser | Thr | Ala | Gln | Gly | Glu |
|-----|-----|-----|-----|-----|-----|-----|-----|

Fig. 2 (1)

4/30

gaagacccca gtgagtgagc atggtaccaa -295

gccatgcttc tctgctctga cagttgatga -235

gaccttcacc ttccactctg atatctgcac -175

acaaacggct cttgctgagc tgggtgaagca -115

gactgtcatg gatgactttg cacagttcct -55

ctgggccagg tcccggccgg cgcc -1

CTT GCA TTG CTG CTG CTG CTG CTC 48

Leu Ala Leu Leu Leu Leu Leu Leu 16

CGC GCC GAG GAA GTC ACT CTA ATG 96

Arg Ala Glu Glu Val Thr Leu Met 32

CTG GGC TGG CTT CTG GAT CCC CCA 144

Leu Gly Trp Leu Leu Asp Pro Pro 48

Fig. 2 (II)

5/30

GAG ACT GGG TGG AGT GAG GTG CAA
Glu Thr Gly Trp Ser Glu Val Gln

TAC ATG TAC CAA GAC TGC CCA ATA
Tyr Met Tyr Gln Asp Cys Pro Ile

TGG CTT CGC TCC AAT TGG ATC TAC
Trp Leu Arg Ser Asn Trp Ile Tyr

TAC GTG GAG CTG CAG TTC ACC GTG
Tyr Val Glu Leu Gln Phe Thr Val

GGA GCT GGG CCT CTG GGA TGC AAA
Gly Ala Gly Pro Leu Gly Cys Lys

GAG AGT GAC CAG GAT GTG GGC ATT
Glu Ser Asp Gln Asp Val Gly Ile

AAG GTA ACA ACT GTG GCA GCA GAC
Lys Val Thr Thr Val Ala Ala Asp

Fig. 2 (III)

6/30

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| CAA | ATG | CTA | AAC | GGG | ACA | CCC | CTG | 192 |
| Gln | Met | Leu | Asn | Gly | Thr | Pro | Leu | 64 |
| CAG | GAA | GGT | GGG | GAC | ACT | GAC | CAC | 240 |
| Gln | Glu | Gly | Gly | Asp | Thr | Asp | His | 80 |
| CGC | GGA | GAG | GAA | GCT | TCA | CGC | ATC | 288 |
| Arg | Gly | Glu | Glu | Ala | Ser | Arg | Ile | 80 |
| CGG | GAC | TGT | AAG | AGT | TTC | CCA | GGG | 336 |
| Arg | Asp | Cys | Lys | Ser | Phe | Pro | Gly | 112 |
| GAG | ACC | TTC | AAC | CTT | TTC | TAC | ATG | 384 |
| Glu | Thr | Phe | Asn | Leu | Phe | Tyr | Met | 128 |
| CAG | CTC | CGA | CGA | CCT | TTG | TTC | CAA | 432 |
| Gln | Leu | Arg | Arg | Pro | Leu | Phe | Gln | 144 |
| CAG | AGC | TTC | ACC | ATC | AGA | GAC | CTG | 480 |
| Gln | Ser | Phe | Thr | Ile | Arg | Asp | Leu | 160 |

Fig. 2 (IV)

7/30

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|
| GCA | TCT | GAC | TCT | GTA | AAG | CTG | AAT |
| Ala | Ser | Asp | Ser | Val | Lys | Leu | Asn |
| CTC | ACC | CGC | CGT | GGC | CTC | TAC | TTA |
| Leu | Thr | Arg | Arg | Gly | Leu | Tyr | Leu |
| GTG | GCG | CTA | GTG | TCT | GTA | AGG | GTG |
| Val | Ala | Leu | Val | Ser | Val | Arg | Val |
| GTG | CAT | GGC | TTG | GCC | CAC | TTC | CCT |
| Val | His | Gly | Leu | Ala | His | Phe | Pro |
| TTG | GTT | GAA | GTA | GCT | GGA | ACG | TGC |
| Leu | Val | Glu | Val | Ala | Gly | Thr | Cys |
| GGG | TCC | TCA | GGT | ACA | CCA | CGA | ATG |
| Gly | Ser | Ser | Gly | Thr | Pro | Arg | Met |
| CTG | GTG | CCT | GTG | GGT | CAG | TGC | CAG |
| Leu | Val | Pro | Val | Gly | Gln | Cys | Gln |

Fig. 2 (V)

8/30

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GTA | GAA | CGC | TGC | TCG | TTG | GGC | CAC | 528 |
| Val | Glu | Arg | Cys | Ser | Leu | Gly | His | 176 |
| GCT | TTC | CAC | AAC | CCG | GGT | TCC | TGT | 576 |
| Ala | Phe | His | Asn | Pro | Gly | Ser | Cys | 192 |
| TTC | TAC | CAG | CGC | TGT | GCC | GAG | ACC | 624 |
| Phe | Tyr | Gln | Arg | Cys | Ala | Glu | Thr | 208 |
| GAC | ACT | CTC | CCT | GGA | CCT | GGA | GGG | 672 |
| Asp | Thr | Leu | Pro | Gly | Pro | Gly | Gly | 224 |
| CTC | TCC | CAT | GCA | CAG | ATC | AGC | TTG | 720 |
| Leu | Ser | His | Ala | Gln | Ile | Ser | Leu | 240 |
| CAC | TGC | AGC | CCT | GAT | GGC | GAG | TGG | 768 |
| His | Cys | Ser | Pro | Asp | Gly | Glu | Trp | 256 |
| TGC | GAG | CCT | GGC | TAT | GAA | GAA | AGC | 816 |
| Cys | Glu | Pro | Gly | Tyr | Glu | Glu | Ser | 272 |

Fig. 2 (VI)

9/30

AGT GGA AAT GTG GGA TGC ACT GCC
Ser Gly Asn Val Gly Cys Thr Ala

GAC ATG AAT ACA CTC CGT TGT CTC
Asp Met Asn Thr Leu Arg Cys Leu

GAG TCT GAG GGG TCT ACC ATC TGT
Glu Ser Glu Gly Ser Thr Ile Cys

GCC CCT GGG GAG GGT CCC CAG GTA
Ala Pro Gly Glu Gly Pro Gln Val

CCC CAA AAT CTG AGC TTC TCC ACA
Pro/Gln/Asn/Leu/Ser/Phe/Ser/Thr/

TGG GAG CCC CCC AGA GAT ACA GGG
Trp/Glu/Pro/Pro/Arg/Asp/Thr/Gly/

GTG GAG TGC TTG CAG TGT CGG GGC
Val/Glu/Cys/Leu/Gln/Cys/Arg/Gly/

Fig. 2 (VII)

10/30

| | | | | | | | | |
|---------------------------------|-----|-------------------------|-----|-----|-----|-----|-----|------|
| TGT | CCT | ACT | GGT | TTC | TAT | CGA | GTG | 864 |
| Cys | Pro | Thr | Gly | Phe | Tyr | Arg | Val | 288 |
| AAG | TGC | CCC | CAA | CAT | AGC | ATA | GCA | 912 |
| Lys | Cys | Pro | Gln | His | Ser | Ile | Ala | 304 |
| ACC | TGT | GAG | AAT | GGA | CAT | TAT | CGA | 960 |
| Thr | Cys | Glu | Asn | Gly | His | Tyr | Arg | 320 |
| GCA | TGC | ACA | CGT | CCC | CCA | TCG | GCT | 1008 |
| Ala | Cys | Thr/Arg/Pro/Pro/Ser/Ala | | | | | | 336 |
| TCA | GGG | ACT | CAA | CTC | TCC | CTG | CGC | 1056 |
| Ser/Gly/Thr/Gln/Leu/Ser/Leu/Arg | | | | | | | | 352 |
| GGA | CGC | CAT | GAT | ATC | AGA | TAC | AGC | 1104 |
| Gly/Arg/His/Asp/Ile/Arg/Tyr/Ser | | | | | | | | 368 |
| ATT | GCA | CAG | GAT | GGG | GGT | CCC | TGC | 1152 |
| Ile/Ala/Gln/Asp/Gly/Gly/Pro/Cys | | | | | | | | 384 |

Fig. 2 (VIII)

11/30

CAA CCC TGT GGA AAA GGT GTG CAC

Gln/Pro/Cys/Gly/Lys/Gly/Val/His/

ACC ACA TCT ACC GTG CAA GTG CAA

Thr/Thr/Ser/Thr/Val/Gln/Val/Gln/

ACA TTT ACC GTC AAA TCC CAA AAC

Thr/Phe/Thr/Val/Lys/Ser/Gln/Asn/

AGC CCT AGC AGC GCC TCC CTC AGT

Ser/Pro/Ser/Ser/Ala/Ser/Leu/Ser/

CTC TCT GGC CTG TCA CTG AAG CTG

Leu/Ser/Gly/Leu/Ser/Leu/Lys/Leu/

GAG CTG ACT TGG GCA GGG TCC CGA

Glu/Leu/Thr/Trp/Ala/Gly/Ser/Arg/

AGC TAT GAG CTG CAC GTG CTG AAT

Ser/Tyr/Glu/Leu/His/Val/Leu/Asn/

Fig. 2 (IX)

12/30

| | |
|---------------------------------|------|
| TTT TCC CCG GCT GCT TCC GGG CTC | 1200 |
| Phe/Ser/Pro/Ala/Ala/Ser/Gly/Leu | 400 |
| GGC CTC GAG CCT TAC GCC AAC TAC | 1248 |
| Gly/Leu/Glu/Pro/Tyr/Ala/Asn/Tyr | 416 |
| AGA GTG TCA GGA CTG GAC AGT TCC | 1296 |
| Arg/Val/Ser/Gly/Leu/Asp/Ser/Ser | 432 |
| ATC AAC ATG GGG CAC GCA GAG TCA | 1344 |
| Ile Asn Met Gly His Ala Glu Ser | 448 |
| GTG AAG AAA GAA CCG AGG CAG CTG | 1392 |
| Val/Lys/Lys/Glu/Pro/Arg/Gln/Leu | 464 |
| CCC CGA AAT CCT GGA GGG AAT CTG | 1440 |
| Pro/Arg/Asn/Pro/Gly/Gly/Asn/Leu | 480 |
| CAG GAC GAA GAA TGG CAC CAG ATG | 1488 |
| Gln/Asp/Glu/Glu/Trp/His/Gln/Met | 496 |

Fig. 2 (X)

13/30

GTG TTG GAA CCC AGG GTC TTG CTG

Val/Leu/Glu/Pro/Arg/Val/Leu/Leu/

TAC ATT GTC AGA GTG CGA ACA CTG

Tyr/Ile/Val/Arg/Val/Arg/Thr/Leu/

TCC CCT GAC CAT GAG TTT CGG ACA

Ser/Pro/Asp/His/Glu/Phe/Arg/Thr

ACC GGA GGA GAG ATT GTG GCC GTC

Thr Gly Gly Glu Ile/Val/Ala/Val/

GCT CTG CTG ATC GGG ATT TAT GTC

Ala/Leu/Leu/Ile/Gly/Ile/Tyr/Val/

CAG AGA CAG CAG AGG CAG CGT GAA

Gln Arg Gln Gln Arg Gln Arg Glu

GAC AAG CTG TGG CTA AAA CCC TAT

Asp Lys Leu Trp Leu Lys Pro Tyr

Fig. 2 (XI)

14/30

| | |
|---------------------------------|------|
| ACA AAA CTT CAG CCA GAT ACC ACA | 1536 |
| Thr/Lys/Leu/Gln/Pro/Asp/Thr/Thr | 512 |
| ACC CCA CTG GGG CCT GGC CCT TTC | 1584 |
| Thr/Pro/Leu/Gly/Pro/Gly/Pro/Phe | 528 |
| AGC CCA CCA GTT TCC AGA AGC CTG | 1632 |
| Ser Pro Pro Val Ser Arg Ser Leu | 544 |
| ATC TTT GGA TTG CTG CTT GGA ATA | 1680 |
| Ile/Phe/Gly/Leu/Leu/Leu/Gly/Ile | 560 |
| TTC CGT TCA AGG AGA GGC CAG AGA | 1728 |
| Phe Arg Ser Arg Arg Gly Gln Arg | 576 |
| CGC ACC ACC AAT GTC GAT CGA GAG | 1776 |
| Arg Thr Thr Asn Val Asp Arg Glu | 592 |
| GTG GAC CTC CAG GCC TAT GAG GAC | 1824 |
| Val Asp Leu Gln Ala Tyr Glu Asp | 608 |

Fig. 2 (XII)

15/30

| | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|
| CCT | GCA | CAG | GGA | GCC | TTA | GAC | TTT |
| Pro | Ala | Gln | Gly | Ala | Leu | Asp | Phe |
| CTG | ATT | GTG | GAC | ACT | GTC | ATA | GGA |
| Leu | Ile | Val | Asp | Thr | Val | Ile | Gly |
| CGG | GGA | GCC | CTG | AGA | CTC | CCC | AGC |
| Arg | Gly | Ala | Leu | Arg | Leu | Pro | Ser |
| AAG | ACC | TTG | AAA | GAT | ACA | TCC | CCA |
| Lys | Thr | Leu | Lys | Asp | Thr | Ser | Pro |
| CGA | GAG | GCA | ACT | ATC | ATG | GGC | CAG |
| Arg | Glu | Ala | Thr | Ile | Met | Gly | Gln |
| CTA | GAA | GGT | GTC | ATC | ACA | AAA | AGA |
| Leu | Glu | Gly | Val | Ile | Thr | Lys | Arg |
| TTT | ATG | GAA | AAT | GGA | GCC | CTG | GAT |
| Phe | Met | Glu | Asn | Gly | Ala | Leu | Asp |

Fig. 2 (XIII)

16/30

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|------|
| GCC | CAG | GAA | CTG | GAC | CCA | GCC | TGG | 1872 |
| Ala | Gln | Glu | Leu | Asp | Pro | Ala | Trp | 624 |
| GAA | GGG | GAG | TTT | GGT | GAA | GTG | TAT | 1920 |
| Glu | Gly | Glu | Phe | Gly | Glu | Val | Tyr | 640 |
| CAA | GAT | TGC | AAG | ACT | GTG | GCC | ATT | 1968 |
| Gln | Asp | Cys | Lys | Thr | Val | Ala | Ile | 656 |
| GAT | GGC | TAC | TGG | TGG | AAT | TTC | CTT | 2016 |
| Asp | Gly | Tyr | Trp | Trp | Asn | Phe | Leu | 672 |
| TTC | AAC | CAC | CCA | CAC | ATT | CTA | CGC | 2064 |
| Phe | Asn | His | Pro | His | Ile | Leu | Arg | 688 |
| AAG | CCC | ATC | ATG | ATC | ATC | ACA | GAA | 2112 |
| Lys | Pro | Ile | Met | Ile | Ile | Thr | Glu | 704 |
| GCC | TTT | CTG | AAG | GAA | CGG | GAG | GAC | 2160 |
| Ala | Phe | Leu | Lys | Glu | Arg | Glu | Asp | 720 |

Fig. 2 (XIV)

17/30

CAA CTA GCT CCT GGT CAG CTA GTG
Gln Leu Ala Pro Gly Gln Leu Val

GGC ATG AAC TGC CTC AGT GGC CAC
Gly Met Asn Cys Leu Ser Gly His

GCC AGG AAC ATC TTG GTG AAT CAG
Ala Arg Asn Ile Leu Val Asn Gln

TTT GGC TTG ACC CGC CTC CTG GAT
Phe Gly Leu Thr Arg Leu Leu Asp

CAG GGA GGA AAG ATC CCC ATC CGA
Gln Gly Gly Lys Ile Pro Ile Arg

CAT CGG ATC TTC ACC ACA GCC AGT
His Arg Ile Phe Thr Thr Ala Ser

ACG TGG GAG GTG TTG AGT TTT GGC
Thr Trp Glu Val Leu Ser Phe Gly

Fig. 2 (XV)

18/30

| | | | | | | | | |
|-----|-----|-----|-----|------------|------------|------------|------------|------|
| GCT | ATG | CTA | CTG | GGC | ATA | GCA | TCA | 2208 |
| Ala | Met | Leu | Leu | Gly | Ile | Ala | Ser | 736 |
| AAT | TAT | GTC | CAT | AGA | GAC | CTG | GCT | 2256 |
| Asn | Tyr | Val | His | Arg | <u>Asp</u> | <u>Leu</u> | <u>Ala</u> | 752 |
| AAC | CTG | TGC | TGC | AAG | GTG | TCT | GAC | 2304 |
| Asn | Leu | Cys | Cys | Lys | Val | Ser | Asp | 768 |
| GAC | TTT | GAC | GGC | ACC | TAT | GAA | ACC | 2352 |
| Asp | Phe | Asp | Gly | Thr | Tyr | Glu | Thr | 784 |
| TGG | ACA | GCC | CCA | GAA | GCT | ATT | GCC | 2400 |
| Trp | Thr | Ala | Pro | <u>Glu</u> | Ala | Ile | Ala | 800 |
| GAT | GTG | TGG | AGC | TTT | GGG | ATT | GTA | 2448 |
| Asp | Val | Trp | Ser | Phe | Gly | Ile | Val | 816 |
| GAC | AAA | CCC | TAT | GGG | GAG | ATG | AGC | 2496 |
| Asp | Lys | Pro | Tyr | Gly | Glu | Met | Ser | 832 |

Fig. 2 (XVI)

19/30

AAC CAA GAG GTA ATG AAA AGC ATT
Asn Gln Glu Val Met Lys Ser Ile

CCT GTG GAC TGT CCT GCC CCT CTC
Pro Val Asp Cys Pro Ala Pro Leu

GCT TAC GAT CGT GCC CGT CGA CCC
Ala Tyr Asp Arg Ala Arg Arg Pro

CTG GAA CAG TTG CTT ACT GAC CCC
Leu Glu Gln Leu Leu Thr Asp Pro

TTT GAC CCT AGG GTG ACC TTA CGC
Phe Asp Pro Arg Val Thr Leu Arg

GGG ATC CCT TAT CGA AGT GTC TCT
Gly Ile Pro Tyr Arg Ser Val Ser

AAG CGC TAC ATC CTG CAC TTC CGT
Lys Arg Tyr Ile Leu His Phe Arg

Fig. 2 (XVII)

20/30

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|------|
| GAA | GAT | GGG | TAC | CGG | TTG | CCC | CCT | 2544 |
| Glu | Asp | Gly | Tyr | Arg | Leu | Pro | Pro | 848 |
| TAT | GAA | CTC | ATG | AAG | AAC | TGC | TGG | 2592 |
| Tyr | Glu | Leu | Met | Lys | Asn | Cys | Trp | 864 |
| CAC | TTC | CTC | CAG | CTG | CAG | GCA | CAT | 2640 |
| His | Phe | Leu | Gln | Leu | Gln | Ala | His | 880 |
| CAT | TCC | CTA | AGG | ACA | ATT | GCC | AAC | 2688 |
| His | Ser | Leu | Arg | Thr | Ile | Ala | Asn | 896 |
| CTG | CCC | AGC | CTG | AGT | GGC | TCT | GAT | 2736 |
| Leu | Pro | Ser | Leu | Ser | Gly | Ser | Asp | 912 |
| GAG | TGG | CTT | GAA | TCC | ATA | CGC | ATG | 2784 |
| Glu | Trp | Leu | Glu | Ser | Ile | Arg | Met | 928 |
| TCG | GCT | GGG | CTG | GAC | ACC | ATG | GAG | 2832 |
| Ser | Ala | Gly | Leu | Asp | Thr | Met | Glu | 944 |

Fig. 2 (XVIII)

21/30

TGT GTG CTG GAG CTG ACG GCT GAG
Cys Val Leu Glu Leu Thr Ala Glu

TTG CCA GGG CAC CAG AAA CGA ATT
Leu Pro Gly His Gln Lys Arg Ile

GAC TGAGCATCCA CTGAAAAGAT GCTCC
Asp *

Aggacggggt acagtcaact ccctgggcct

Tgctgctcct gcccagtcaa tcagaactct

Aggggggtgga tgggtacaag tgaaggggnc

natacactta catatgcatt atctatTTTT

Aaaaaaaaaag gaattcctgc agcccggggg

Ggtggagctc cagcttttTgt tcccttta

Fig. 2 (XIX)

22/30

| | |
|----------------------------------|------|
| GAC CTG ACG CAG ATG GGA ATA ACG | 2880 |
| Asp Leu Thr Gln Met Gly Ile Thr | 960 |
| CTC TGC AGT ATT CAA GGA TTT AAG | 2928 |
| Leu Cys Ser Ile Gln Gly Phe Lys | 976 |
| AGCCC TCTGCCTGCC TCCATTAGCA | 2981 |
| | 977 |
| ttcctcagcc tacgaaatgt aggctattgg | 3041 |
| gcctttgaac caaggagcct ttgtttataa | 3101 |
| tgtgggtggg ttctggggga gggtttaata | 3161 |
| gtaaataaac aagagttgag ttttaaaaaa | 3221 |
| atccactagt tctagagcgg ccgccaccgc | 3281 |
| | 3309 |

Fig. 2 (XX)

23/30

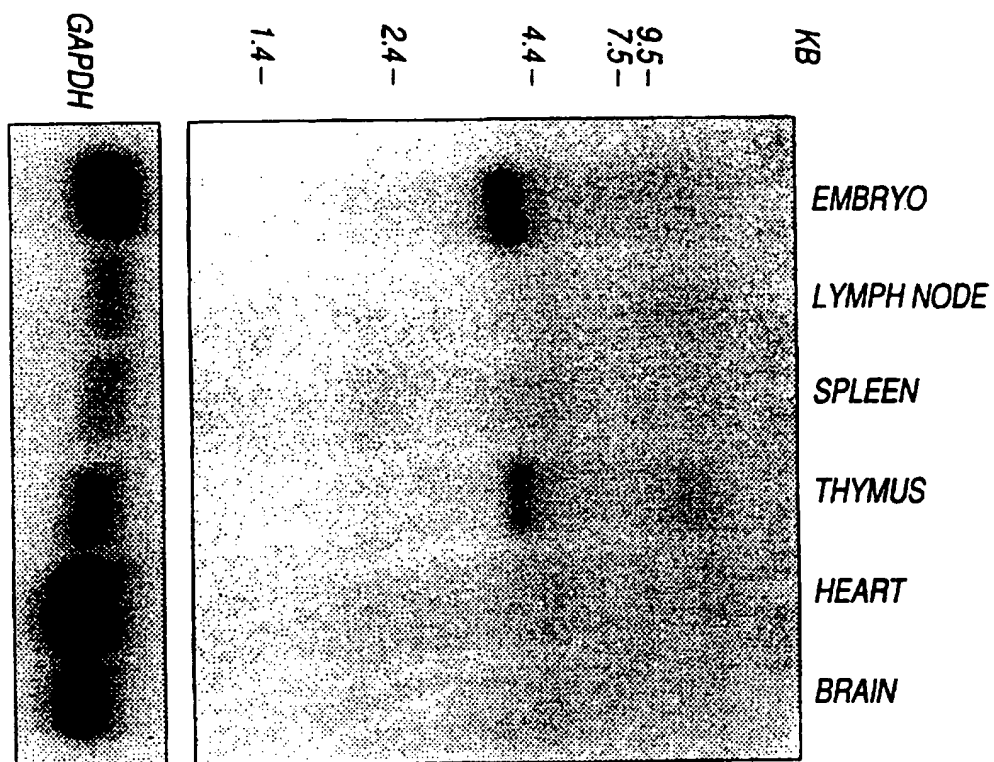
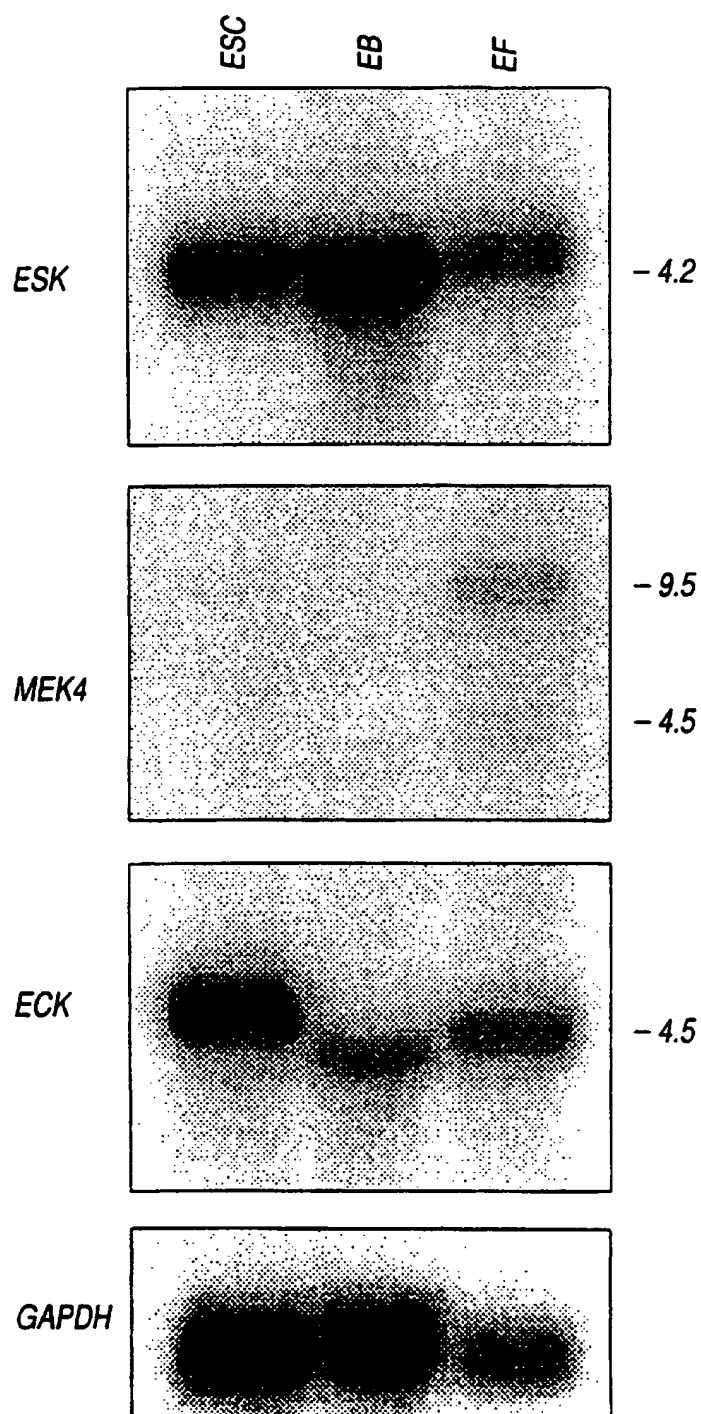


Fig. 3

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*Fig. 4*

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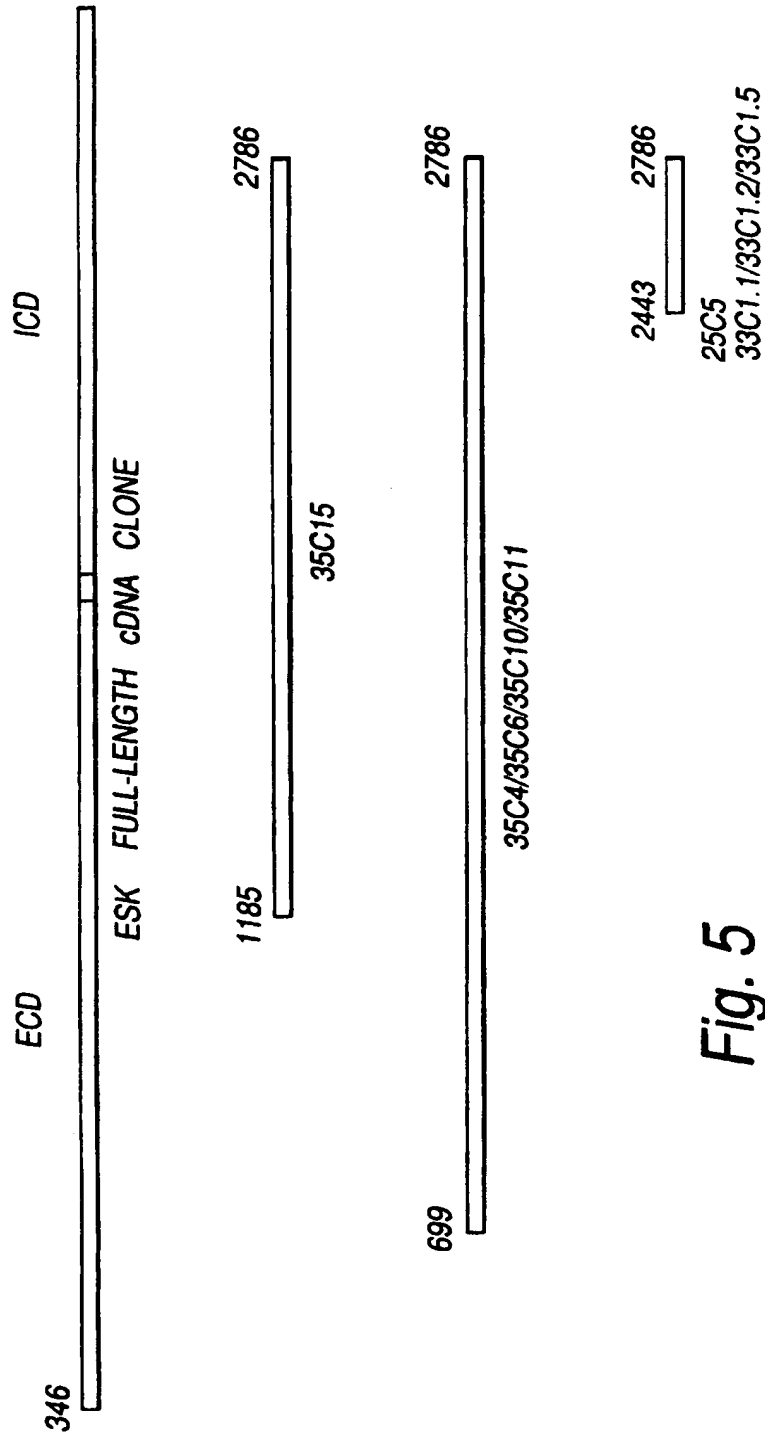
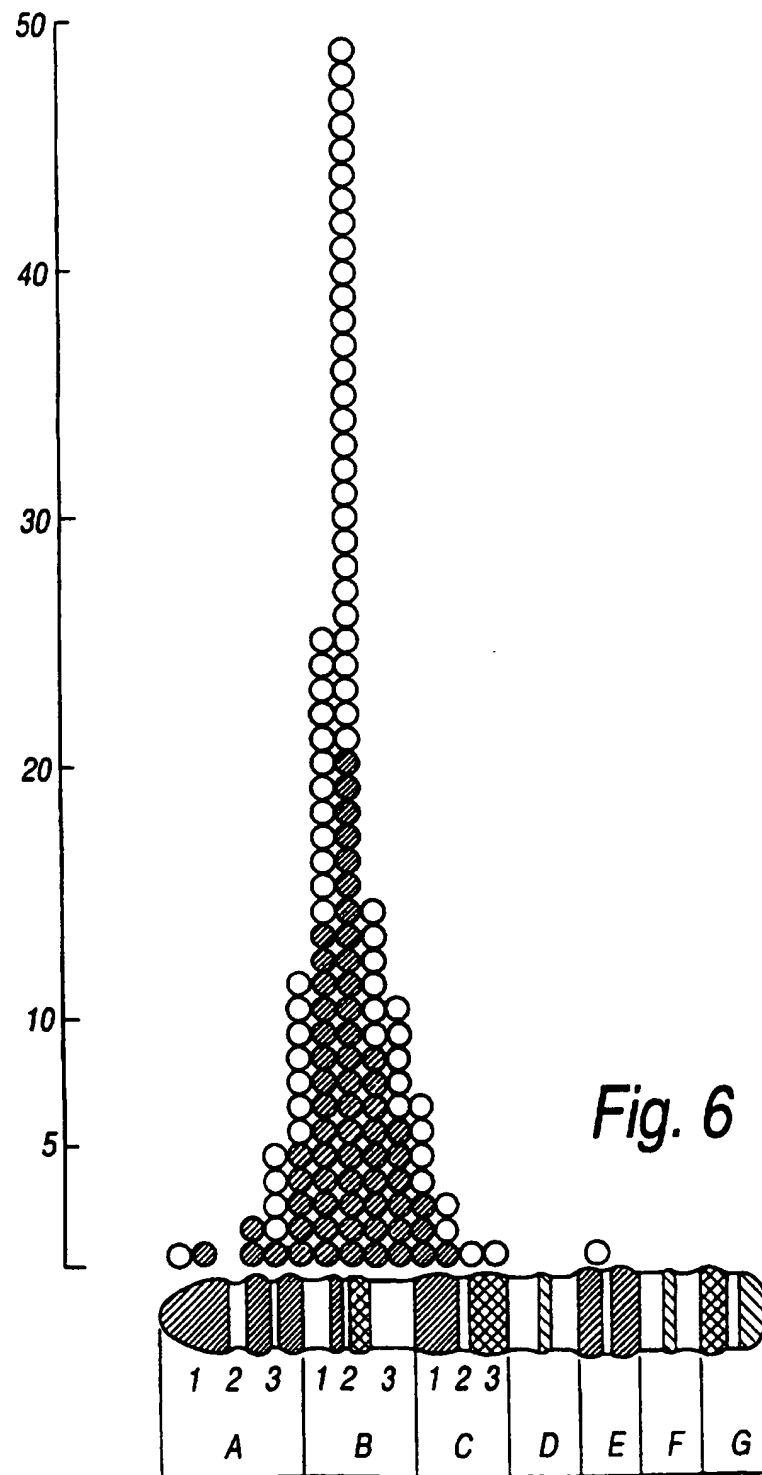
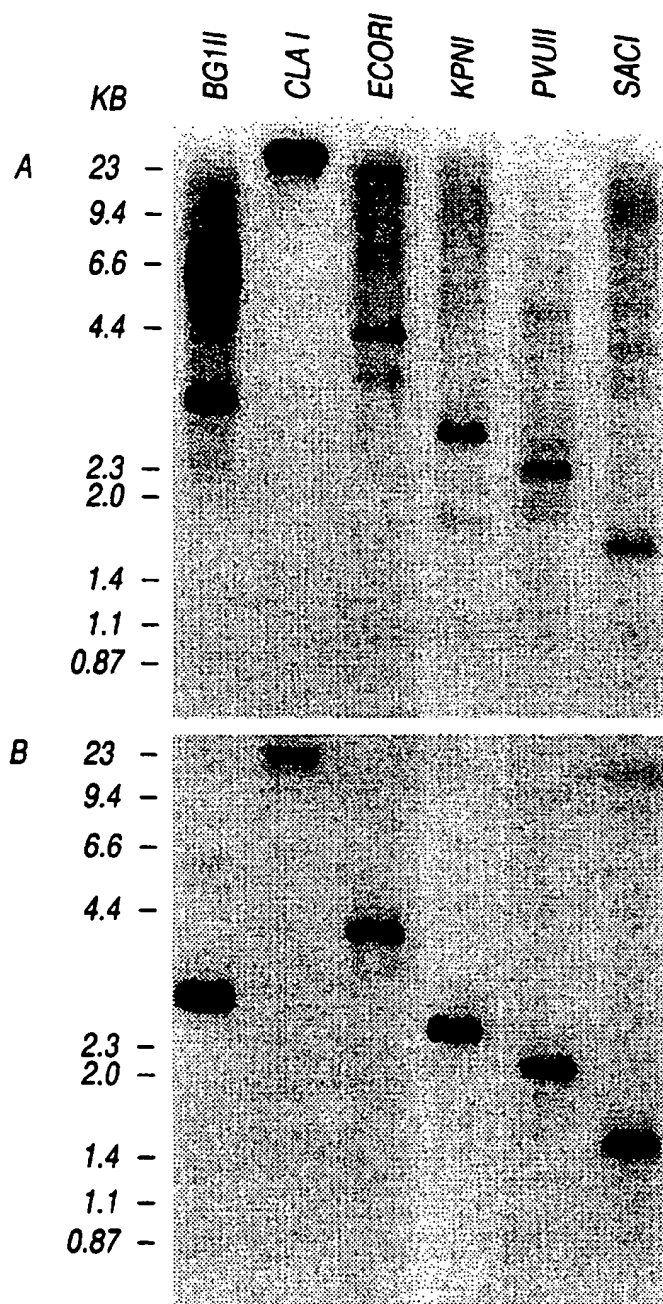


Fig. 5

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27/30

*Fig. 7*

28/30

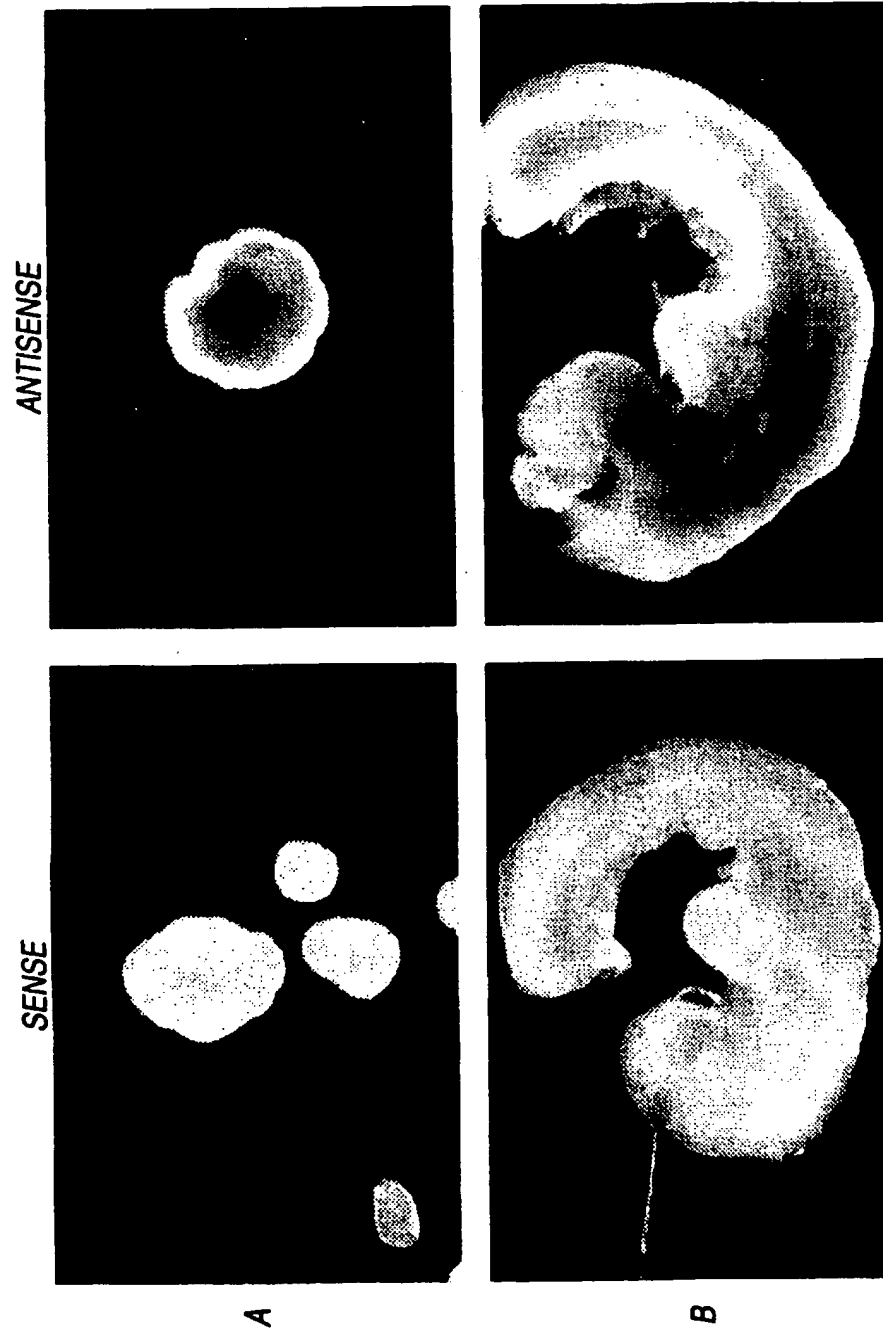


Fig. 8

29/30

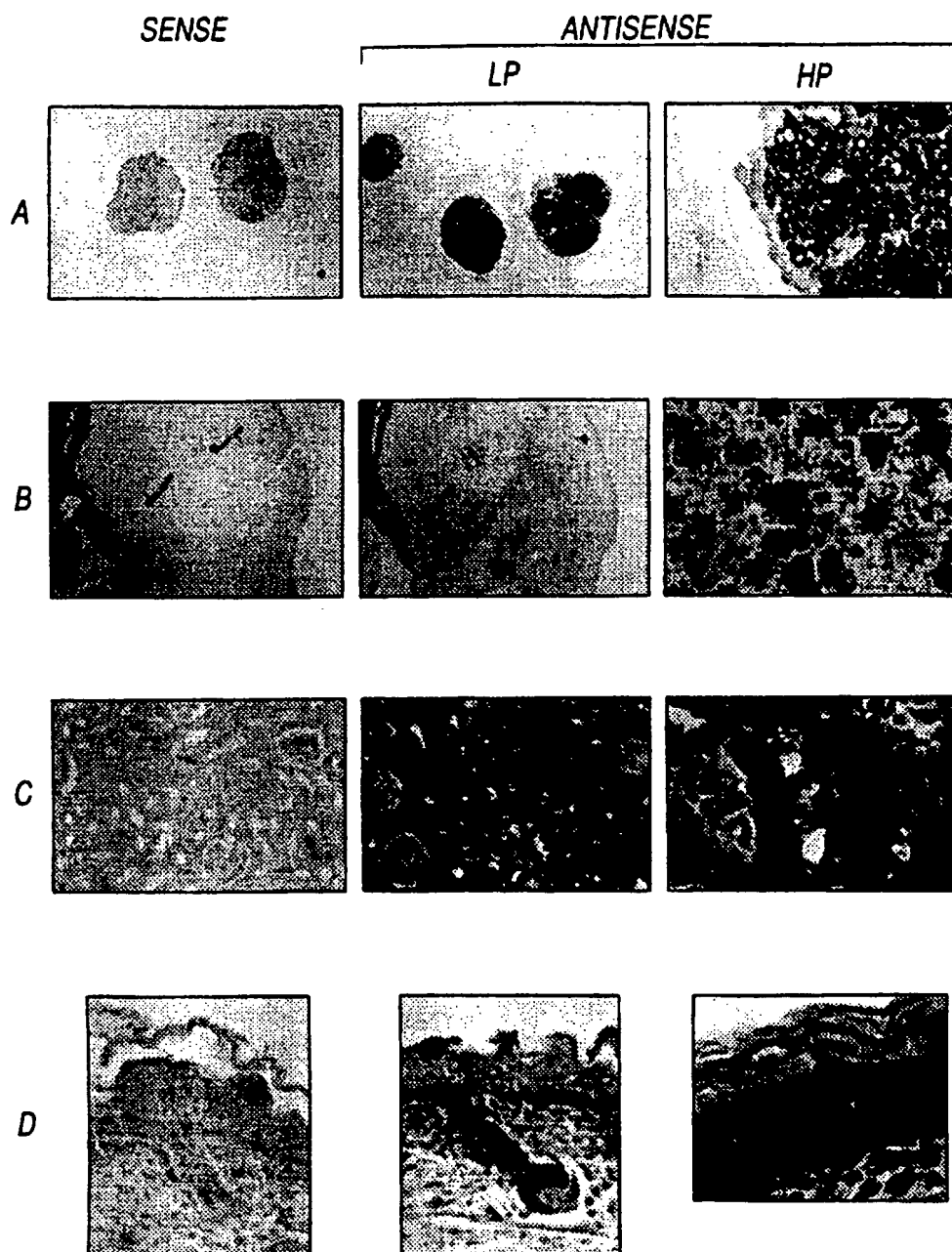


Fig. 9

30/30

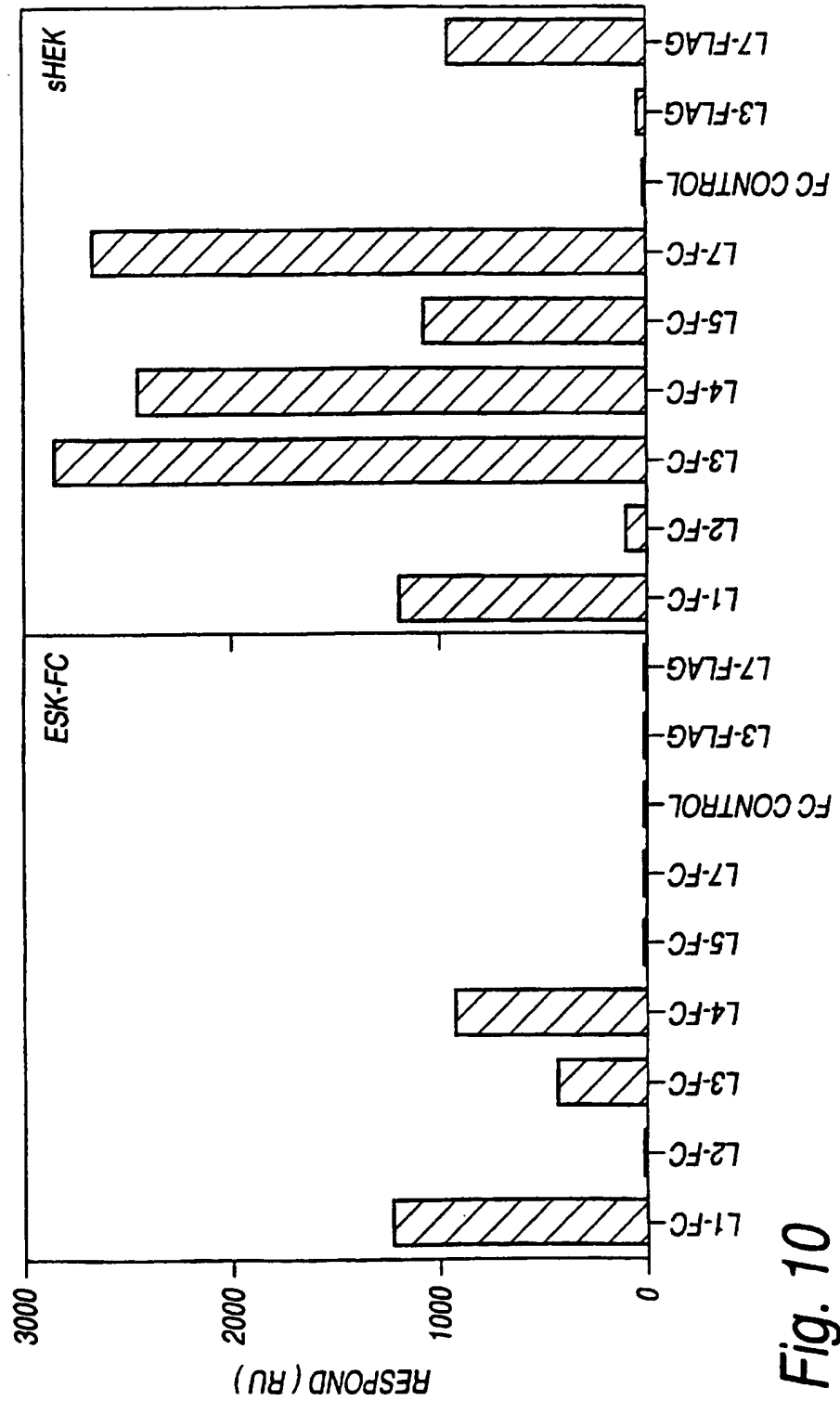
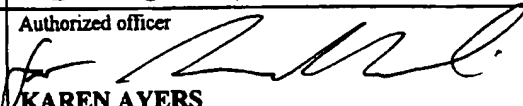


Fig. 10

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00826

| | | | | |
|---|---|--|--|---|
| A. CLASSIFICATION OF SUBJECT MATTER | | | | |
| Int Cl ⁶ : C12N 15/54; C07K 14/71, 16/28; A61K 38/17, 39/395 | | | | |
| According to International Patent Classification (IPC) or to both national classification and IPC | | | | |
| B. FIELDS SEARCHED | | | | |
| Minimum documentation searched (classification system followed by classification symbols) | | | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Medline: Receptor protein-tyrosine kinase and EPH Angis: full peptide sequence SEQ I.D. No.2 | | | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) | | | | |
| See continuation | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | |
| P,X | Proc. Natl. Acad. Sci. USA. Vol 93, pp 145-150, (January 1996), Lickliter et al. "Embryonic stem cells express multiple Eph-subfamily receptor tyrosine kinases" (see whole document) | 1-20 | | |
| X | Science, vol. 238 pp 1717-1720, (1987), Hirai et al. "A novel putative tyrosine kinase receptor encoded by the <u>eph</u> gene" (see whole document) | 1-20 | | |
| A | Embo Journal, vol 14(13) pp 3116-3126 (1995), Brambilla et al. "Membrane-bound LERK 2 ligand can signal through three different Eph-related receptor tyrosine kinases". (see whole document) | 1-20 | | |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input type="checkbox"/> See patent family annex | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </td> </tr> </table> | | | <p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> | <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> |
| <p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> | <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> | | | |
| Date of the actual completion of the international search 13 February 1997 | | Date of mailing of the international search report 20 FEB 1997 | | |
| Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929 | | Authorized officer  KAREN AYERS Telephone No.: (06) 283 2082 | | |

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00826

Box continuation of Box B

STN: Amino acid sequence

ECLQCRGI|AASGLTTSTVQVQ/SQSP

WPAT/USPM/JAPIO:

RTK OR RPTK OR RECEPTOR (W) PROTEIN (W) TYROSINE (W) KINASE# OR RECEPTOR (W)
TYROSINE (W) KINASE#

CAS online:

L1 RTK OR RPTK OR RECEPTOR (W) PROTEIN (W) TYROSINE(W) KINASE# OR RECEPTOR (W)
TYROSINE (W) KINASE#

L2 EPH

L3 L1 AND L2

L6 EPH (5A) RELATED

L7 L6 AND L3

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/AU 96/00826

| C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | Oncogene, vol. 10, pp 897-905 (1995), Fox et al., "cDNA cloning and tissue distribution of five human EPH-like receptor protein-tyrosine kinases" (see whole document) | 1-20 |